

Hyperparasitism by *Sphaerellopsis macroconidialis* may lower over-wintering survival of *Uromycladium acaciae*

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Abstract

Uromycladium acaciae has caused an epidemic of wattle rust on *Acacia mearnsii* in southern Africa since 2013. In April 2016, conidiomata of a species of *Sphaerellopsis* were observed parasitising telia of *U. acaciae* on foliar samples collected in three plantations in Mpumalanga, South Africa. An isolate was identified as *Sphaerellopsis macroconidialis* based on a phylogenetic species hypothesis of the internal transcribed spacer (ITS) region. This is the first report of this species of mycoparasite from South Africa and on this host. To investigate the seasonal population dynamics of this mycoparasite, the presence of *Sphaerellopsis* on telia of *U. acaciae* was assessed monthly between May 2016 and April 2017. The proportion of samples with conidiomata of *Sphaerellopsis* was greatest between May and November. This period is outside both the peak growing season for *A. mearnsii* and the main epidemic period for *U. acaciae*. Results suggest that *Sphaerellopsis* may have little impact on *U. acaciae* during epidemic periods of the year but could reduce the over-wintering

survival of this damaging rust fungus and lower initial inoculum loads at the start of the following growth season.

Key words: Mycoparasite; Fungi; Biocontrol; Natural Enemy; Forestry

Introduction

Uromycladium acaciae causes a damaging rust disease on *Acacia mearnsii* (black wattle) in southern Africa (Fraser et al. 2020). First observed in the KwaZulu-Natal Midlands in 2013, this disease is now found throughout the KwaZulu-Natal and Mpumalanga Provinces of South Africa, as well as in neighboring Eswatini (formerly Swaziland) (Fraser et al. 2020). *Uromycladium acaciae* produces spermogonia and telia on all above ground plant parts, with symptoms and signs including rachis and rachilla malformation, production of chocolate-brown telia that become “slimy” under wet conditions, matting of leaves, and stunting of growth (Fraser et al. 2020).

Conidiomata of a *Sphaerellopsis* species were observed on telia of *U. acaciae* collected during monitoring of disease development in Mpumalanga in April 2016. Species of *Sphaerellopsis* (synonyms: *Darluca*, *Eudarluca*) are mycoparasites of rust fungi (Trakunyingcharoen et al. 2014) and have been investigated as potential biocontrol agents (Gordon and Pfender 2009). *Sphaerellopsis filum* is the best-known taxon having been reported on a wide range of rust species and from most continents. However, Trakunyingcharoen et al. (2014) showed that this was a species complex and described two additional species, *S. macroconidialis* and *S. paraphysata*.

Two species of *Sphaerellopsis* have been reported from South Africa. Based on morphology, the asexual and sexual stages of *S. filum* (as *Darluca filum* and *Eudarluca*

filum) have been reported from South Africa (Doidge 1950). Severe infections of *Sphaerellopsis* on uredinia of unidentified rust fungi were observed to prevent the development of telia (Doidge 1950). Based on DNA sequence data, Trakunyingcharoen et al. (2014) reported *S. paraphysata* infecting *Ravenelia macowania* on *Vachellia karroo* in South Africa.

Sphaerellopsis filum (as *Darluca filum*) has previously been reported on a gall-forming species of *Uromycladium* (possibly *U. paradoxae*, reported as *U. tepperianum*) on *Acacia stricta* near Sydney (Burgess 1934). Infection by *S. filum* reduced the spore output of the galls by half or more (Burgess 1934).

Knowledge of the biology of *Uromycladium*, including its interaction with mycoparasites such as *Sphaerellopsis*, is limited. The discovery of conidiomata of *Sphaerellopsis* on telia of *U. acaciae* at disease development monitoring sites in South Africa provided an opportunity to gather novel data on the identity and biology of the species of *Sphaerellopsis* present.

Material and Methods

At the start of 2016, three plantations (Dundonald, Iswepe, and Moolman) of *A. mearnsii* infected by *U. acaciae* in Mpumalanga were selected for monitoring of disease symptom development (see Fraser et al. 2020). All sites were in the south east of Mpumalanga near the border with Eswatini, an area with a temperate climate characterised by dry winters and wet summers. Wattle rust was monitored on three randomly selected trees within each stand. After the first observation of *Sphaerellopsis* in April 2016, samples of up to ten leaves with symptoms from the most recent growth were collected monthly until April 2017 from each tree. These were inspected under a dissection microscope (Discovery.V12, Zeiss) for the presence or absence of

conidiomata of *Sphaerellopsis*. The proportion of leaves with conidiomata of *Sphaerellopsis* at each time point was calculated to investigate seasonal patterns of abundance.

The identity of an isolate (CBS 147187) of *Sphaerellopsis* obtained from the Dundonald plantation in April 2016 was investigated using a phylogenetic species concept based on a sequence of the internal transcribed spacer region (ITS) of ribosomal DNA (Fig. 1). Methods for fungal isolation, DNA extraction and PCR were the same as those described in Trakunyingcharoen et al. (2014). The ITS sequences of CBS 147187 and *S. hakeae* were added to the alignment of Trakunyingcharoen et al. (2014) and the most likely tree identified using the IQTree web server (Trifinopoulos et al. 2016). Intraspecific diversity in the ITS region for species of *Sphaerellopsis* was visualised using a minimum spanning network (Bandelt et al. 1999) sampled from all available ITS sequences on GenBank.

Results and Discussion

The ITS sequence of isolate CBS 147187 (MT998445) obtained from the Dundonald plantation in April 2016 was identified as *Sphaerellopsis macroconidialis* based on a phylogenetic species hypothesis. It was 98.9% (533 out of 539 nucleotides) identical to the ex-type sequence of *S. macroconidialis* (CBS 658.78, KP170659), and formed a monophyletic group with isolates identified by Trakunyingcharoen et al. (2014) and the type sequence of *S. hakeae* (Fig. 1). There was intraspecific variation in the ITS region of *S. macroconidialis* with 1–3 parsimony informative characters between haplotypes in the minimum spanning network (Fig. 1).

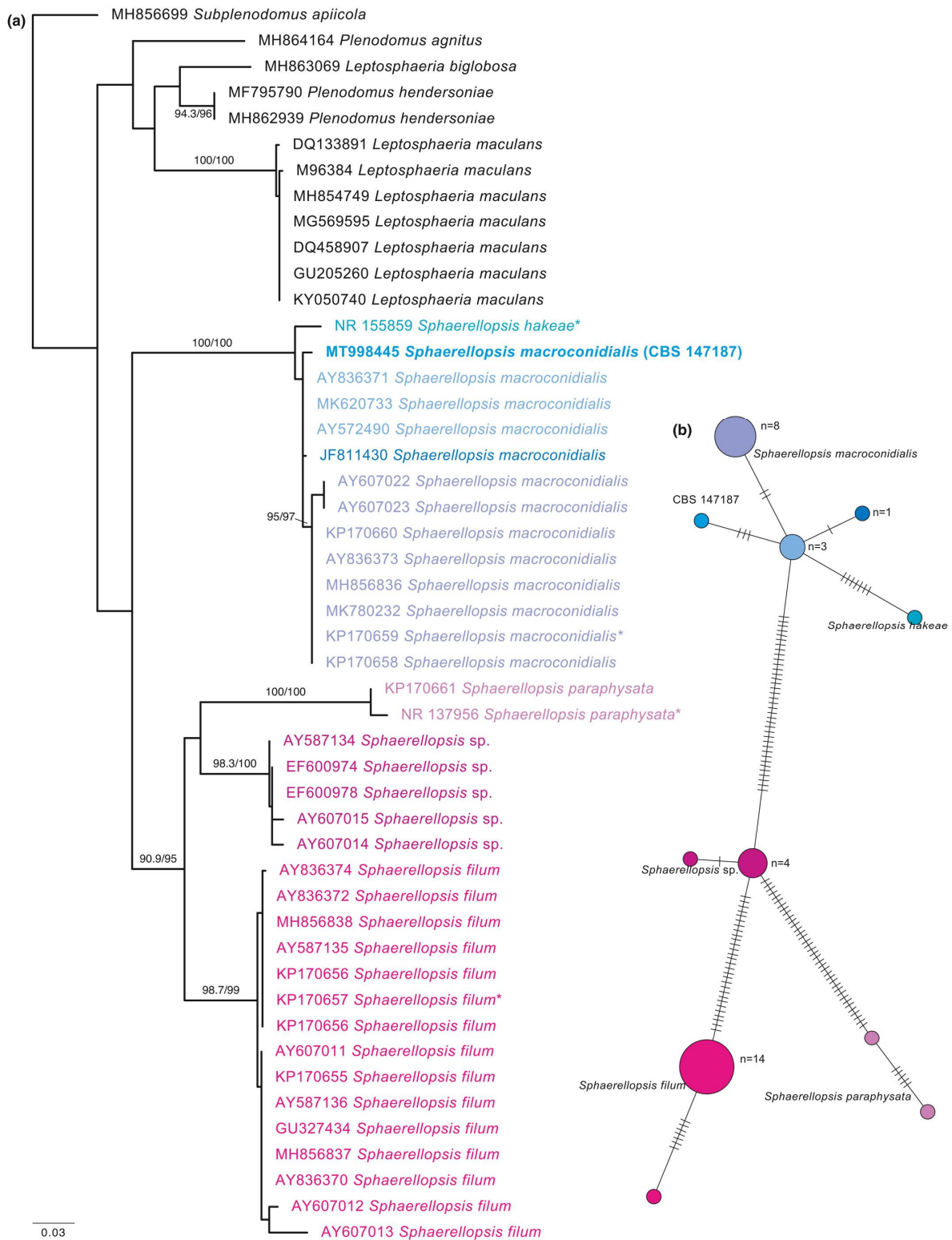


FIGURE 1. (a) Phylogram obtained from a maximum likelihood analysis of the ITS region of rDNA in IQTree v.1.7 beta. aRLT values ($\geq 90\%$) and ultrafast bootstrap values ($\geq 95\%$) from 10,000 replicates above nodes. Ex-type sequences are marked with an asterisk. (b) Minimum spanning network sampled from all available ITS sequences of *Sphaerellopsis* on GenBank (including sequences labelled as *Eudarluc*a). Colours of each circle correspond to taxa in the phylogram, and hashes indicate number of parsimony-informative sites in the alignment. The taxon on *Uromycladium acaciae* (CBS 147187) was identified as *S. macroconidialis* based on a phylogenetic species concept

This is the first report of *S. macroconidialis* from South Africa and also the first time the mycoparasite has been found infecting *Uromycladium acaciae*. The fungus has previously been known only from Europe on *Uromyces caryophylli*, *Puccinia allii* and an unidentified rust on *Carex acutiformis* (Trakunyingcharoen et al. 2014). We have applied *S. macroconidialis* as a name to all collections of *Sphaerellopsis* on telia of *U. acaciae*, although a molecular barcode was obtained from only one isolate. There may be greater species diversity of these mycoparasites on *U. acaciae* with increased taxonomic sampling, but this was not the focus of the present study.

Conidiomata were observed mainly on older telia of *U. acaciae*. Conidiomata were observed at every timepoint on samples from Iswepe, however, at the other two sampling sites, there were time points with no detections, particularly in the summer months. Seasonal patterns of observations of the mycoparasite were similar at all three sites, with greater abundance during the dry season between May and November (Fig. 2).

Periods of greater abundance of *S. macroconidialis* were outside the peak growing season for *A. mearnsii* and the main epidemic period for *U. acaciae* (Fraser et al. 2020). Foliage and rust pustules collected during this period were therefore older. This pattern of occurrence corresponds with the observation of conidiomata mostly on older telia. This pattern and behaviour may limit the impact of the mycoparasite on *U. acaciae* during the epidemic period of the year when there is a greater abundance of fresh telia. However, *S. macroconidialis* may affect the over-wintering survival of telia and reduce the inoculum load at the start of the following growing season. This has been shown in related systems; for example *Sphaerellopsis filum* has been shown to reduce the over-wintering population of *Puccinia graminis* subsp. *graminicola* (Gordon and Pfender 2012).

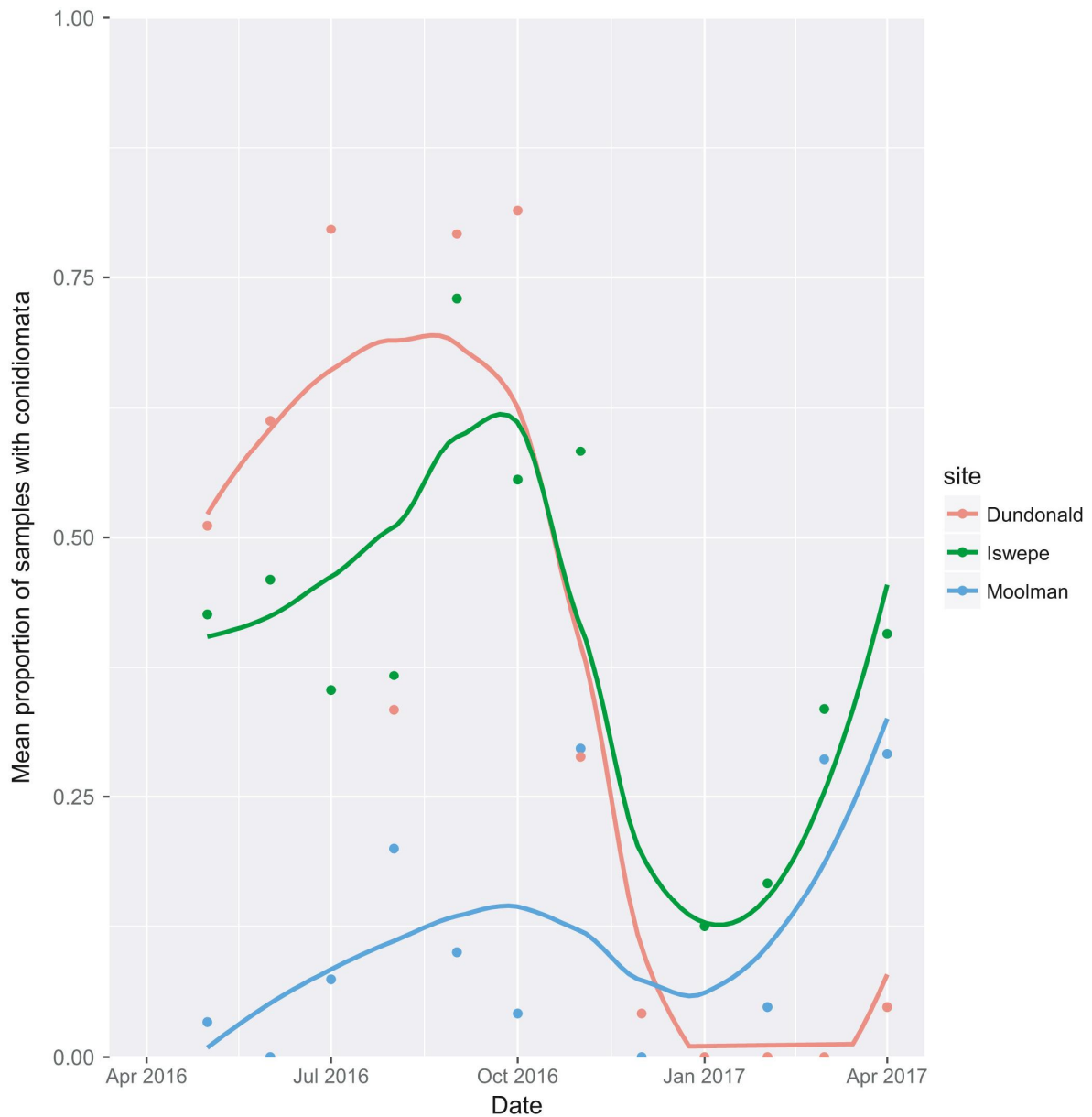


FIGURE 2. Seasonal detection of conidiomata of *Sphaerellopsis macroconidialis* on telia of *Uromycladium acaciae* on the freshest growth of *Acaciamearnsii* at three plantations in Mpumalanga, South Africa. Trend lines were fitted with locally weighted smoothing (LOESS)

This study has identified *S. macroconidialis* as a hyperparasite of *U. acaciae*. It increases the known host range of the mycoparasite and is the first report of this fungus from South Africa. We have determined that *S. macroconidialis* is more abundant after the optimal growth conditions of *U. acaciae* and hypothesise that it does not significantly impact disease severity in epidemic periods. But, it may reduce over-

wintering survival and therefore initial inoculum load in the following season. Further research should consider the impact of *S. macroconidialis* on wattle rust severity and evaluate its potential as a biocontrol agent.

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