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Mites are the most common vectors of the fungus *Gondwanamyces proteae* in *Protea* infructescences

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ABSTRACT

Entomochoric spore dispersal is well-documented for most ophiostomatoid fungal genera, most of which are associated with bark or ambrosia beetles. *Gondwanamyces* spp. are unusual members of this group that were first discovered in the flower heads of the primitive angiosperm genus *Protea*, that is mostly restricted to the Cape Floristic region of Africa. In this study, we present the discovery of the vectors of *Gondwanamyces proteae* in *Protea repens* infructescences, which were identified using PCR, direct isolation, and light microscopy. *Gondwanamyces proteae* DNA and ascospores were identified on diverse lineages of arthropods including beetles (*Euderes lineicolis* and *Genuchus hottentottus*), bugs (*Oxycarenus maculates*), a psocopteran species and five mite (Acari) species. Based on isolation frequency, however, a mite species in the genus *Trichouropoda* appears to be the most common vector of *G. proteae*. *Gondwanamyces* spores were frequently observed within pit mycangia at the base of the legs of these mites. Manipulative experiments demonstrated the ability of mites to carry viable *G. proteae* spores whilst in transit on the beetle *G. hottentottus* and that these mites are able to transfer *G. proteae* spores to uncolonised substrates *in vitro*. Interestingly, this same mite species has also been implicated as vector of *Ophiostoma* spores on *P. repens* and belongs to the same genus of mites that vector *Ophiostoma* spp. associated with pine-infesting bark beetles in the Northern Hemisphere.

Introduction

The ophiostomatoid fungi (Wingfield *et al.* 1993) include species that reside in two distantly related orders, the Ophiostomatales and the Microascales (Hausner *et al.* 1992; 1993a; 1993b; Spatafora & Blackwell 1994). Species in these orders have undergone convergent evolution that promotes entomochoric spore dispersal and are often treated collectively due to their morphological and ecological similarities, despite a lack of phylogenetic relatedness (Spatafora & Blackwell 1994). Ophiostomatoid fungi generally produce flask-shaped sexual structures with extended necks (teleomorph) or stalk-like asexual structures (anamorph) that bear sticky spore droplets at their tips. These exposed spores are ideally positioned to easily adhere to, and be dispersed by, arthropods (Münch 1907; 1908; Francke-Grosmann 1967; Whitney 1982; Beaver 1989; Malloch & Blackwell 1993; Cassar & Blackwell 1996).

The ophiostomatoid fungi have a global distribution, but are best known in the Northern Hemisphere. In this region they are typically associated with the galleries of bark and ambrosia beetles (Coleoptera: Curculionidae, Scolytinae), which are particularly well-studied on conifers (Francke-Grosmann

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1967; Upadhyay 1981; Whitney 1982; Christiansen et al. 1987; Wingfield et al. 1993; Paine et al. 1997; Kirisits 2004). The group includes a number of primary plant pathogens and many agents of timber degradation (Münch 1907; Upadhyay 1981; Whitney 1982; Sinclair et al. 1987; Seifert et al. 1993; Jacobs & Wingfield 2001).

A unique and unusual assemblage of saprobic ophiostomatoid fungi occurring in the flower heads (infructescences) of the African endemic plant genus *Protea* was first discovered during the latter part of 20th century (Wingfield et al. 1988). Currently, 11 species in two genera, *Ophiostoma* (Ophiostomatales) and *Gondwanamyces* (Microascales), are known only from the senescent infructescences of these plants. *Ophiostoma* is the more speciose genus in this niche, including seven South African species; *Ophiostoma africanum*, *Ophiostoma gemellus*, *Ophiostoma protearum*, *Ophiostoma splendens*, *Ophiostoma palmiculminatum*, *Ophiostoma phasma*, and the anamorphic *Sporothrix variecibatus* (Marais & Wingfield 1994; 1997; 2001; Roets et al. 2006a; 2008). Two additional species, *Ophiostoma protea-sedis* and *Ophiostoma zambiensis* were recently added from *Protea caffra* in Zambia (Roets et al. 2010), confirming the distribution of *Protea*-associated *Ophiostoma* into tropical and sub-tropical Africa (Roets et al. 2009b). In addition to these, numerous other South African *Ophiostoma* spp. have been described from very diverse habitats, including soil, wooden utility poles used to support overhead power lines (de Meyer et al. 2008), and wounds on native trees (Kamgan et al. 2008).

In contrast to *Ophiostoma*, only two species of the teleomorph genus *Gondwanamyces* have been described from Africa (*Gondwanamyces proteae* and *Gondwanamyces capensis*). These species are known only from the infructescences of *Protea* species in the Western Cape Province of South Africa. *Gondwanamyces proteae* appears to be specific to its *Protea repens* host, while *G. capensis* has been collected from many different *Protea* spp. (Roets et al. 2009b). A third species, *Gondwanamyces scolytodes*, is associated with the ambrosia beetle *Scolytodes unipunctatus* (Coleoptera: Curculionidae, Scolytinae) on the plant host *Cecropia angustifolia* in mountain cloud rainforests of Costa Rica (Kolarik & Hulcr 2009).

Gondwanamyces spp. are characterised by *Custingophora* anamorphs, cycloheximide sensitivity and a phylogenetic position inferred from rDNA sequences (Marais et al. 1998; Viljoen et al. 1999; Kolarik & Hulcr 2009). Two *Custingophora* spp. are thought to be closely related to *Gondwanamyces* (Kolarik & Hulcr 2009); *Custingophora olivacea* which was described from compost in Germany (Stolk & Hennebert 1968), and *Custingophora cecropiae* is known as associate of *S. unipunctatus* on *C. angustifolia* (Kolarik & Hulcr 2009). The phylogenetic relatedness of these two species to the other four species in *Custingophora* is unknown (Pinnoi et al. 2003; Kolarik & Hulcr 2009).

Like other ophiostomatoid fungi, those associated with *Protea* were presumed to be entomochoric. This was based on general morphology with ascospores and conidia produced in sticky drops on the tips of elongated structures (Wingfield et al. 1988; Wingfield & Van Wyk 1993; Marais & Wingfield 1994). More recent studies have confirmed the association of *Protea*-colonising *Ophiostoma* with arthropods (Roets et al. 2006c; 2007; 2008). Various mites (*Proctolaelaps vandenbergi*, two *Tarsonemus* spp. and a *Trichouropoda* sp.) play a primary role in the dissemination of *Ophiostoma* spores on *Protea* (Roets et al. 2007). In addition, the *Trichouropoda* sp. has a mutualistic association with its phoretic *Ophiostoma* spp. It was thus shown that this mite is able to complete its life cycle on a diet consisting solely of *Ophiostoma* spp. In a subsequent study, Roets et al. (2009a) demonstrated that long-range dispersal of the mites and their associated *Ophiostoma* spp. is achieved through a phoretic association with the beetles *Genuchus hottentottus*, *Trichostetha fascicularis*, and *Trichostetha capensis*.

In contrast to *Ophiostoma*, nothing is known regarding the dispersal of *Protea*-associated *Gondwanamyces* spp. The closely related species *C. cecropiae* and *G. scolytodes* have been collected from *Scolytodes* and are presumed to be dispersed by these beetles (Kolarik & Hulcr 2009). Other closely related fungi in the genus *Ceratocystis* (Microascales) are vectored by various insects (Iton 1966; Moller & DeVay 1968; Hinds 1972; Kile 1993) including bark beetles (Yamaoka et al. 1997; Harrington & Wingfield 1998; Krokene & Solheim 1998). However, no bark or ambrosia beetles are known to be associated with *Protea* spp. Based on the morphological similarity between *Gondwanamyces* and *Ophiostoma*, it is reasonable to suspect that *Gondwanamyces* spp. have vectors similar to those for *Ophiostoma* spp. occurring on its *Protea* hosts.

The aim of this study was to evaluate various arthropods as potential dispersal agents for *Gondwanamyces* on *Protea*. The investigation is focussed specifically on *G. proteae* that occurs exclusively on *P. repens* individuals, because this fungus has ascospores with a very distinct shape that cannot easily be confused with other fungal species from this niche.

Materials and methods

Arthropod collection

Three hundred, 1-y-old *Gondwanamyces proteae*-colonised *Protea repens* infructescences were collected from the Jonkershoek Forestry Reserve, Stellenbosch in the Western Cape Province of South Africa (33°58.591′S 18°56.817′E) between Jan. 2003 and Aug. 2008. Infructescences were placed in emergence cages (Roets et al. 2007) at room temperature and all arthropod individuals that emerged over a 40-d-period were collected at 3 d intervals. This method allowed for the collection of large arthropods (up to 4 mm) only. In order to collect smaller organisms such as mites, a number of additional *G. proteae*-colonised infructescences (*ca.* 100) were opened directly after collection from the field. Individuals of small species were then extracted using a fine camel-hair brush and a dissecting needle.

The surfaces of larger arthropods were cleared of debris and phoretic arthropods using a dissecting needle. All collected arthropods were classified into morpho-species and stored at −20 °C until further analysis. Voucher specimens are maintained in the insect collection (USEC), Department of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, South Africa (Table 1).

Vector identification using polymerase chain reaction (PCR)

Molecular identification of putative *Gondwanamyces* vectors followed methods described by Roets et al. (2007). In that study, arthropods were tested for the presence of *Ophiostoma* DNA using

Table 1 – Arthropods collected from the infructescences of *Protea repens* and tested for the presence of (1) *Gondwanamyces* DNA using PCR techniques and (2) *Gondwanamyces* reproductive propagules using plating techniques. Species in bold were found to carry *Gondwanamyces* DNA and/or reproductive propagules. Numbers between parentheses indicate number of individuals that tested positive using a particular technique.

Arthropod taxa	Ref. nr	Number of individuals		
		Tested using PCR	Tested using plating	Total (%)
INSECTS				
Argyroploce sp. Hübner (Tortricidae)	68	7	1	0.00
Curculionidae sp.	48	4	4	0.00
Diptera sp.	5	12	8	0.00
***Euderes lineicolis* Wiedemann (Curculionidae)**	**33**	**9**	**14 (1)**	**4.35**
Formicidae (sp. 2)	56	9	20	0.00
***Genuchus hottentottus* (F) (Scarabaeidae)**	**70**	**28 (3)**	**121 (6)**	**3.60**
Histeridae	32	7	1	0.00
Nitidulidae	25	30	19	0.00
***Oxycarenus maculates* Stal. (Lygaeidae)**	**7**	**51**	**100 (1)**	**0.01**
Psocoptera (sp. 1)	31	12	100	0.00
Psocoptera (sp. 2)	**12**	**4**	**100 (1)**	**0.01**
Psocoptera (sp. 3)	13	66	100	0.00
Sphenoptera Solier sp. (Buprestidae)	49	2	1	0.00
Staphylinidae sp.	35	1	6	0.00
Tinea sp. L. (Tineidae)	67	4	1	0.00
MITES				
***Ameroseius proteaea* Ryke (Ameroseiidae)**	**M1**	**18**	**250 (8)**	**0.03**
***Trichouropoda* sp. Berlese (Uropodidae)**	**M2**	**23**	**400 (56)**	**13.24**
***Tarsonemus* sp.**	**M13**	**0**	**250 (5)**	**0.02**
Lorryia sp. Oudemans (Tydeidae)	M3	1	173	0.00
Tenuelamellarea hispanica Subias & Itor. (Lamellareidae)	M4	3	198	0.00
***Humerobates setosus* Behan-Pelletier & Mahunka (Humerobatidae)**	**M5**	**2**	**250 (1)**	**0.00**
Bdellodes sp. Oudemans (Bedellidae)	M6	1	111	0.00
***Proctolaelaps vandenbergi* Ryke (Ascidae)**	**M7**	**14**	**250 (1)**	**0.00**
Zygoribatula setosa Evans (Oribatulidae)	M8	2	14	0.00

a PCR protocol developed by Roets *et al.* (2006c). In this study, we evaluated all arthropods collected from *Protea repens* and tested them for the presence of *Gondwanamyces* DNA using similar methods. The same total genomic DNA extracted from macerated individuals (Roets *et al.* 2007) was used in the current study to test for amplification of *Gondwanamyces* DNA.

Expected product length after amplification of *Gondwanamyces* DNA with the primers GPR1 (Roets *et al.* 2006c) and LR6 (Vilgalys & Hester 1990) was *ca.* 640 bp. To verify positive amplification results, PCR products of this length were cleaned (Wizard® SV gel and PCR clean-up system, Promega, Madison, Wisconsin, U.S.A.) and sequenced (Big Dye™ Terminator v3.0 cycle sequencing premix kit, Applied Biosystems, Foster City, CA, U.S.A) with an ABI PRISIM™ 3100 Genetic Analyser (Applied Biosystems).

Vector identification by direct plating of arthropods

To verify the putative vectors for *Gondwanamyces proteae*, individuals collected from emergence cages and directly from infructescences were tested for the presence of *Gondwanamyces* reproductive propagules using techniques described by Roets *et al.* (2007). Individuals were crushed and, depending on the size of the arthropod, vortexed in 2–10 ml ddH_2O. Suspensions were plated on Petri dishes (1 ml/plate) containing 2 % malt extract agar [(MEA), Biolab, Midrand, South Africa] emended with streptomycin sulphate (0.04 g L^{-1}).

Plates were incubated at 25 °C in the dark and inspected at 48 hourly intervals for fungal growth. Colonies of *Gondwanamyces*, recognised in culture by their *Custingophora* anamorphs are very slow-growing (Wingfield *et al.* 1988; Wingfield & Van Wyk 1993). All colonies of non-*Gondwanamyces* isolates were thus cut from the agar in Petri dishes using a scalpel as soon as these appeared. Arthropod individuals were recorded as non-carriers of *Gondwanamyces* reproductive propagules where no colonies of this genus appeared after 4 weeks of incubation. The presence and identity of putative *Gondwanamyces* isolates (as *Custingophora* asexual states) were determined using colony- and microscopic fungal characteristics. One *Gondwanamyces* colony per arthropod individual was randomly chosen as a representative culture for the collection. Representative cultures were deposited in the culture collection (STE-U) of the Department of Plant Pathology, Stellenbosch University, South Africa.

Vector identification by light microscopy

Approximately 500 individuals of the suspected primary vector (a mite species in the genus *Trichouropoda*) were collected from *Protea repens* infructescences heavily colonised by *Gondwanamyces proteae*, from the Jonkershoek Nature Reserve. These arthropods were mounted on microscope slides in lactophenol containing cotton blue. Mounts were heated over an open flame for 10 s and the position of fungal spores was identified

using a Nikon Eclipse E600 light microscope with differential interference contrast. Microscopic examinations focused on detecting ascospores that have a distinct morphology. This is in contrast to the conidia formed by *Custingophora* spp. that are easily confused with those of other fungal taxa. Ascospores of *G. proteae* are one celled, hyaline, fusiform with a distinct falcate hyaline gelatinous sheath, 7–13 × 2–4 μm and they tend to stick together (Wingfield *et al.* 1988). Photographic images were captured using a Nikon DXM1200 digital camera.

Dispersal of Gondwanamyces proteae

Gondwanamyces proteae-colonised *Protea repens* infructescences ($n = 100$) that also showed insect borer damage were collected from Gordon's Bay (34°04′58.80′S 21°15′20.52′E) during Oct. 2010 and placed in emergence cages as described by Roets *et al.* (2007). Boxes were left open and placed in a dark room 6 M from an uncovered, closed window. A shallow tray (5 cm deep) and sized to fit the window sill was placed in front of the window. As the infructescences dried, individuals of *Genuchus hottentottus* (Scarabeidae: Coleoptera; one of the main vectors of *Protea* infructescence-associated mites identified in Roets *et al.* (2009a)) that emerged, flew to the window and landed in the tray. Individuals of *G. hottentottus* were collected from the tray on a daily basis over a 3-week-period. When present, individuals of *Trichouropoda* sp. mites were aseptically removed with a fine dissecting needle and individually stored at 4 °C in Eppendorf tubes. Collected mites were processed within 2 d after collection. Mites were crushed, mixed with 1 ml ddH_2O and plated onto 2 % MEA plates amended with streptomycin sulphate (0.04 g L^{-1}). Plates were periodically inspected for the presence of *Custingophora* colonies over a 3-week-period.

Inoculation of MEA with Gondwanamyces proteae *by mites*

Gondwanamyces proteae-colonised *Protea repens* infructescences were collected from the Jonkershoek Forestry Reserve during Jul. 2009 and Oct. 2010. *Trichouropoda* sp. mites were collected from these using methods described in Roets *et al.* (2009a). Briefly these methods entail the collection of mites that move freely from infructescences, up plant stems and into artificially constructed 'infructescences' (darkened glass vials containing moist filter paper shreds) under desiccating conditions. Individuals of the collected mites were allowed to move freely on Petri dishes containing 2 % MEA emended with 0.04 g L^{-1} streptomycin sulphate (one mite per dish). The experiment was replicated 250 times. In order to confirm the presence of *G. proteae* spores on the collected mites, an additional 131 mites were crushed, mixed with 1 ml ddH_2O and plated onto 2 % MEA plates emended with streptomycin sulphate (0.04 g L^{-1}). Petri dishes were kept at 24 °C in the dark and regularly inspected for the presence of *Custingophora* colonies for 5 weeks.

Results

Arthropod collection

Various arthropod morpho-species collected by Roets *et al.* (2007) and in this study were excluded from further analyses as they were considered unlikely to act as primary vectors of *Gondwanamyces proteae*. These included all spiders and arthropod morpho-species for which less that 10 individuals were collected in total. These arthropods were considered 'tourists' on *Protea repens* and were thus unlikely to be specifically associated with this plant species. A total of 24 arthropod morpho-species were finally included in the analyses (Table 1).

Vector identification using PCR

Four arthropod individuals (three morpho-species) produced DNA amplicons of *ca.* 640 bp in length using the newly developed PCR method (Roets *et al.* 2006c). Sequencing of these DNA fragments, however, revealed that only *Genuchus hottentottus* carried *Gondwanamyces* DNA (Table 1). Consistent with the study of Roets *et al.* (2007) for the amplification of *Ophiostoma* DNA from arthropods, this PCR method did not exclusively amplify DNA of *Gondwanamyces*. It did, however, allow for the rapid screening of a large number of individuals for putative *Gondwanamyces* vectors.

Vector identification by direct plating of arthropods

Only 3 % of tested arthropod individuals ($n = 2679$) yielded cultures of *Gondwanamyces proteae* using plating techniques (Table 1). These putative vector morpho-species were taxonomically very diverse and included beetles (e.g. *Euderes lineicolis* and *Genuchus hottentottus*), a bug (e.g. *Oxycarenus maculates*), a psocopteran species and five mite species. The frequency of morpho-species individuals found carrying spores of *G. proteae* was, however, generally very low (0.01–0.03 %). Individuals of the two beetles, *E. lineicolis* and *G. hottentottus*, carried reproductive propagules of *G. proteae* at intermediate frequencies (4.35 % and 3.60 %, respectively).

In contrast to other morpho-species tested, many individuals of the *Trichouropoda* sp. mite carried *G. proteae* reproductive propagules. *Gondwanamyces proteae* isolates were obtained from more than 13 % of all individuals ($n = 456$) of this mite species (Table 1). *Trichouropoda* sp. mites were found to be common in the larval tunnels of boring insects, e.g. *G. hottentottus* in *Protea repens* infructescences as previously reported by Roets *et al.* 2007. These mites were also commonly observed on the surface of *G. hottentottus* on which they are phoretic.

Vector identification by light microscopy

Based on the results of the plating studies, we focussed on the visual detection of *Gondwanamyces proteae* ascospores on *Trichouropoda* sp. only. Even though the incidence of spore-carrying individuals of this species was fairly high, it was possible only to detect ascospores of *G. proteae* on two wild-caught individuals using light microscopy. In both cases the spores were situated within depressions at the base of the second pair of legs of the mites (Fig 1). Spores of various other unidentified fungal species were also observed in these depressions.

Dispersal of Gondwanamyces proteae

Fourteen individuals of *Genuchus hottentottus* were collected from the emergence cages. Forty-four *Trichouropoda* sp. mites

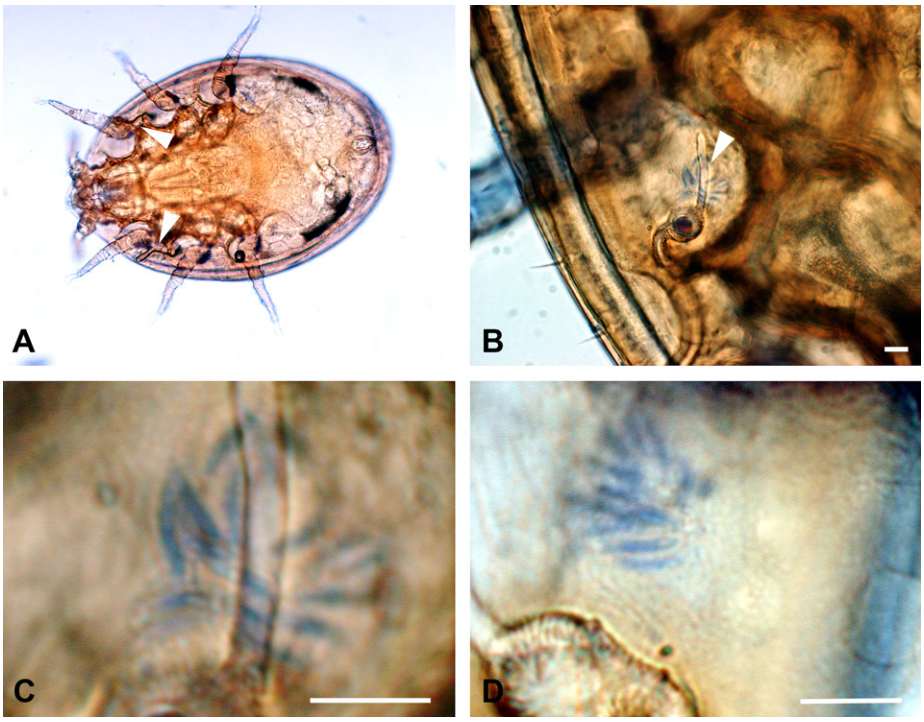

Fig 1 – Ascospores of *Gondwanamyces proteae* on *Trichouropoda* sp. mites collected from *P. repens* infructescences. (A) Ventral side of mite showing location where *G. proteae* spores are generally carried (arrows). (B) *Gondwanamyces proteae* ascospores (arrow) associated with depression at base of legs. (C) Same, enlarged. (D) Ascospores of *G. proteae* on a different mite individual. Scale bars: 10 μm.

were collected from the beetles at an average of 3.14 (±2.41) mites per individual beetle. Mites were usually found at the base of the first pair of legs or in the junction between the head and thorax of the beetles. *Custingophora* colonies were initiated from nine of the mites collected from flying *G. hottentottus* (20.45 % of total mites).

Inoculation of MEA with Gondwanamyces proteae *by mites*

Twenty-one of the 131 *Trichouropoda* sp. mites (16. 03 %) collected from the artificial 'infructescences' and plated revealed the presence of *Custingophora*. Most plates that were inoculated with live individual mites were overgrown with fast growing mould genera (e.g. *Penicillium* and *Cladosporium* spp.) within a week. However, colonies of the slow-growing *Custingophora proteae* were found on five of the 250 plates (0.02 %) occupied by individual mites. This represents a transfer rate of 8.8 %, if we assume that *ca.* 16 % of the individual mites initially transferred to the plates carried *G. proteae* ascospores.

Discussion

Results of this study provide the first evidence that *Protea*-associated *Gondwanamyces* spp. are vectored by arthropods, a prediction made when the first species in this genus (as *Ceratocystiopsis proteae*) was described (Wingfield *et al.* 1988). The results also suggest that the most common vector for *Gondwanamyces proteae* on *Protea repens* is a mite species in the genus *Trichouropoda*. This contrasts with results from recent studies that suggested a close association between *Gondwanamyces* species and *Scolytodes* bark beetles in Costa Rica (Hulcr *et al.* 2007; Kolarik & Hulcr 2009). The discovery of mites as the vectors of *Gondwanamyces* from *Protea* hosts stresses the importance of studying the ecology and evolution of this system in greater depth. For example, it is possible that mites may influence the vectoring of fungal spores in *Scolytodes*-associated *Gondwanamyces*. This discovery also indicates that mites should receive more attention as possible significant vectors of fungal species in other systems that involve fungi, which occupy similar niches to *Gondwanamyces* (e.g. *Ceratocystis* spp.), and where mite associations have not yet been investigated. For example, mites may also play a significant role in the Nitidulid beetle – *Ceratocystis* systems investigated by e.g. Juzwik *et al.* (1998), Cease & Juzwic (2001) and Heath *et al.* (2009).

This study has exposed a remarkable overlap in the arthropods responsible for vectoring *Ophiostoma* and *Gondwanamyces* on *Protea* hosts. Five mite species were identified as vectors of *Gondwanamyces* spores. Three of these (*Proctolaelaps vandenbergi*, the *Tarsonemus* sp. and the *Trichouropoda* sp.) have also been shown to be vectors of *Ophiostoma* from *Protea* (Roets *et al.* 2007). In that study, 14 % of the *Trichouropoda* individuals, 2 % of the *Tarsonemus* individuals, and 0.8 % of the *P. vandenbergi* individuals were found to carry spores of *Ophiostoma* on numerous *Protea* spp. In *P. repens* specifically, only the *Trichouropoda* sp. was found to carry spores of *Ophiostoma*, where 18 % of the tested individuals carried *Ophiostoma* spores. Here we show that this mite species is likely to also be the main vector

for *G. proteae*. Interestingly, fairly similar proportions of these mites carry spores of *Ophiostoma* and *Gondwanamyces* (18 % and 13 % respectively).

In addition to mites, a few individuals of a diverse range of insects (*Euderes lineicolis*, *Genuchus hottentottus*, *Oxycarenus maculates* and a psocopteran species) were found to carry reproductive propagules of *Gondwanamyces*. Similarly, Roets *et al.* (2007) isolated *Ophiostoma* from *P. repens*-associated *G. hottentottus*, *O. maculates* and a different psocopteran species. As noted by Roets *et al.* (2007), when compared to the high numbers of infructescences colonised by *Gondwanamyces* and *Ophiostoma* in the field, these insects are found in *Protea* infructescences at low frequencies (Coetzee & Giliomee 1987a; 1987b; Roets *et al.* 2006b). However, contrary to what has previously been suggested, we believe that the presence of both fungal genera on these insects indicates that they may play a significant role in the dissemination of ophiostomatoid spores in this niche.

Long-range dispersal of *P. vandenbergi*, the *Tarsonemus* sp. and the *Trichouropoda* sp. has been shown to involve phoresy *via* various large beetles (Coleoptera: Scarabaeidae) that frequent *Protea* infructescences (Roets *et al.* 2009a). The *Trichouropoda* sp. mite was, however, only found phoretic on *G. hottentottus*. Combined results of the present study and that of Roets *et al.* (2009a) suggest that the *P. repens*-associated *Gondwanamyces* and *Ophiostoma* spp. are likely to be primarily dispersed by the *Trichouropoda* sp., while the *G. hottentottus* beetles play a secondary role. *Protea*-associated *Ophiostoma* and *Gondwanamyces* thus not only share similar morphologies and the same microhabitat (infructescences), but also have very similar ecologies in terms of their primary and secondary vectors. In addition, these genera have similar seasonal sporulation times, mainly in autumn and winter (Roets *et al.* 2005). The precise mechanisms that enable them to co-inhabit and thrive, usually even within a single infructescence, are still unknown, but merit further study.

Arthropod associations are common among fungi that are phylogenetically allied to *Gondwanamyces*. *Ceratocystis* spp., for example, are vectored by arthropods (Juzwik *et al.* 1998; Cease & Juzwic 2001; Heath *et al.* 2009) with which they usually have fairly loose relationships (Moller & DeVay 1968; Hinds 1972; Kile 1993). Seemingly more specialised species are found associated with bark beetles in their galleries (Redfern *et al.* 1987; Wingfield *et al.* 1997; Yamaoka *et al.* 1997). *Custingophora* spp. have been collected from various organic substrates, including the galleries of bark beetles (Kolarik & Hulcr 2009), suggesting that they may be associated with various arthropods. It would therefore be very interesting to consider whether arthropod-associated fungi in the Microascales generally evolved from loose and casual associations with various arthropods to the more specialised systems involving bark and ambrosia beetles. A possible role of mites in the transmission of spores of *Scolytodis*-associated *Gondwanamyces* should, therefore, also be considered.

The importance of mites as vectors of ophiostomatoid fungi, in general, should not be underestimated. Over 90 species of mites are, for instance, associated with the southern pine beetle *Dendroctonus frontalis*, 14 of which are phoretic on the beetle (Moser & Roton 1971). Many of these phoretic mites are fungivorous, and may thus also carry fungal propagules (Moser & Roton 1971). Amongst the contingent of phoretic mites on *D. frontalis*, species of the genus *Tarsonemus* (*Tarsonemus ips*, *Tarsonemus krantzii*, and *Tarsonemus fusarii*), *Trichouropoda* and *Proctolaelaps* are of special interest, as these same genera are implicated in the *Protea* system. They are not injurious to the beetle while in transit (Moser & Roton 1971), but may impact the beetles indirectly by transporting additional fungal spores (Lombardero *et al.* 2000; Lombardero *et al.* 2003). Similar to the *Tarsonemus* sp. found on *Protea*, the *Tarsonemus* mites from *D. frontalis* possess specialised spore-carrying structures (sporothecae) that have been shown to frequently contain spores of the ophiostomatoid fungi (e.g. *Ophiostoma minus*) (Bridges & Moser 1983; Moser 1985; Moser *et al.* 1995). These mites also have a mutualistic association with their phoretic fungi (Lombardero *et al.* 2000). Mites influence the population dynamics of *D. frontalis* by vectoring *O. minus*, a fungus that limits the success of the beetle mycangial fungi, and consequently lower the success of the beetles (Lombardero *et al.* 2000; Klepzig *et al.* 2001a, 2001b; Lombardero *et al.* 2003). Thus, these associations are very complex and include a communalism (mites and beetles), two mutualisms (mites-fungi and mycangial fungi-beetles) and competition (mite fungi *vs.* beetle mycangial fungi) (Lombardero *et al.* 2003). The influence of ophiostomatoid fungi on the success of *G. hottentottus* on *Protea* is unknown, but may prove to be an interesting field for future study.

The infructescences of *Protea* represent a unusual habitat for ophiostomatoid fungi. In recent years, a suite of studies has added considerably to our understanding of this group of ecologically important fungi. However, much remains to be resolved regarding the ability of *Gondwanamyces* and *Ophiostoma* spp. to occupy seemingly similar ecological niches, simultaneously. It is plausible that other *Protea*-infructescence colonising organisms play a role in niche separation of these genera. In order to clarify this question, studies on the competitive abilities of these fungi in association with other organisms are needed.

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