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RESEARCH ARTICLE

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Association between sub-cortical beetles, mites, and ophiostomatoid fungi on trees in South African Afromontane forests

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Sub-cortical beetles and mites contribute to tree mortality by creating wounds and by spreading potential pathogens. Here we elucidate associations between sub-cortical beetles, mites, and ophiostomatoid fungi from trees in Afromontane forests in South Africa. Bark and wood samples were collected from native tree species and exotic *Pinus radiata* and *Acacia mearnsii* that showed signs of sub-cortical beetle activity, or from wounds on storm-damaged trees. Ophiostomatoid fungi, a group that contains numerous tree pathogens, were isolated from beetles that emerged from samples, their galleries, phoretic mites on these beetles, and from wounds and wound-associated mites. Fungal isolates were identified using multiple DNA markers. Twenty sub-cortical beetle, 22 mite and 16 ophiostomatoid fungal species were recovered from only a few native and exotic host tree species. Three fungal species are likely undescribed despite increased focus on this ecologically and economically important group worldwide. Significantly, some mites and fungi were isolated from native and non-native hosts. Wound-associated fungi and mites were less host-specific than sub-cortical beetle-associated taxa. We highlight the rich and still unexplored symbioses between sub-cortical beetles, mites, and ophiostomatoid fungi in Afromontane forests and provide a foundation for future studies on the ecology of these important organisms.

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INTRODUCTION

Afromontane forests comprise small, fragmented patches within river valleys, on mountains, foothills, and coastal platforms on the eastern side of the African continent, extending from South Africa northwards to the Arabian Peninsula with some isolated patches on the Cameroon highlands on the west coast of Africa. The largest fragments in South Africa are in the Tsitsikamma and Knysna regions, but this biome also extends through the northeastern parts into Eswatini. Afromontane forests are home to a diverse biota that contain many endemic species (De Klerk et al. 2002). Limited research has been conducted on the diversity of potential pests and pathogens present in Afromontane forests (e.g. Machingambi et al. 2015; Van der Colff et al. 2015). Exotic pests and pathogens can cause serious ecological damage (Brasier 2008; Harrington et al. 2008). For example, the recent establishment of the invasive pest *Euwallacea fornicatus* (Coleoptera, Curculionidae, Scolytinae) has the potential to severely degrade Afromontane forests by killing thousands of trees within a relatively short period (de Wit et al. 2022; Townsend et al. 2024).

Sub-cortical beetles are considered some of the most significant forest pests globally (Liebhold et al. 2012). Even when native, these can shape the structure and function of entire forests. For example, many upland forests in the USA are dominated by longleaf and shortleaf pine trees, because the more susceptible loblolly pines are selectively eliminated by the southern pine bark-beetle (Curculionidae, Scolytinae) (Schowalter et al. 1981). In the lowlands, the growth of hardwood trees is more pronounced than in the highlands, which leads to stress on pines due to overcrowding. Stressed pines are more susceptible to attack by the southern pine bark-beetle (Walker 1962), leading to the gradual transformation of lowland pine forests into hardwood forests over time (Schowalter et al. 1981).

Many sub-cortical beetles such as members of the Scolytinae and Platypodinae (Curculionidae) form symbioses with ophiostomatoid fungi (Ascomycota: Ophiostomatales and Microascales) (Six 2003; Whitney 1982). These fungi are represented by well-known genera such as *Ceratocystiopsis* Upadhyay and Kendrick, *Ceratocystis* Ellis and Halst., *Graphium* Corda, *Knoxdaviesia* Wingfield, Van Wyk and Marasas, *Ophiostoma* Sydow and Sydow and *Sporothrix* Hektoen and Perkins (De Beer et al. 2013). Numerous species are important tree pathogens. *Ophiostoma*, for example, includes several virulent pathogens such as the well-known *Ophiostoma ulmi* (Buisman) Nannf. and *O. novo-ulmi* Brasier, responsible for the Dutch elm disease pandemics in North America and Europe (Brasier and Buck 2001). Numerous *Sporothrix* species are widely responsible for sapstain on lumber and pulpwood, leading to high economic losses (Seifert 1993; Zhou et al. 2001). Some *Raffaelea* species are serious plant pathogens, such as *Raffaelea lauricola* T.C. Harr., Fraedrich and Aghayeva, which is responsible for the laurel wilt disease epidemic in the south-eastern USA (Harrington et al. 2008).

A few studies on Afromontane tree pathogens have included ophiostomatoid fungi (e.g. Kamgan Nkuekam et al. 2008). *Ceratocystis tsitsikammensis* Kamgan and Roux, for example, is a wound-infecting fungus that is pathogenic to *Rapanea melanophloeos* (Kamgan Nkuekam et al. 2008). Regular vectors for *Ceratocystis* between wounds on trees in other parts of the world include nitidulid- (Cease and Juzwik 2001; Kamgan Nkuekam et al. 2012b) and bark-beetles (Wingfield et al. 1997; Harrington and Wingfield 1998). However, linkages between these organisms are understudied in Afromontane forests. Also, little is known regarding the diversity and ecology of other ophiostomatoid genera and their associated organisms in Afromontane forests even though numerous species may be of great ecological and economic importance (Musvuugwa et al. 2015, 2016a, 2016b).

Bark- and ambrosia-beetles often gain nutritional benefits from their fungal symbionts (Klepzig & Six 2004; Six & Paine 1998) and in turn transport the fungi from one host tree to the next, suggesting a mutualistic association (Klepzig and Six 2004; Six 2003; Whitney 1982). In some cases, the beetles evolved specialised spore-carrying structures to help maintain and protect the fungi from desiccation and contaminants during transport (Klepzig and Six 2004; Paine and Birch 1983). However, not all ophiostomatoid fungi form symbioses with sub-cortical beetles (Harrington et al. 2008; Hofstetter and Moser 2014) as nitidulid beetles (Kamgan Nkuekam et al. 2012a; 2012b) and even mites (Klepzig et al. 2001a; Moser 1985; Roets et al. 2007) have also been implicated as common symbionts on tree wounds and other micro-habitats. The association between some mites and ophiostomatoid fungi has proven to be mutualistic (Klepzig et al. 2001a; 2001b; Moser 1985; Roets et al. 2007). The mites feed on the fungi and, again, some mite species evolved specialised structures to house fungal spores while in transit between host plants (Bridges and Moser 1983; Hofstetter and Moser 2014; Moser et al, 1995; Roets et al. 2007). The mites are often phoretic on other insects including sub-cortical beetles and even birds, which carry them to un-colonised hosts (Hofstetter and Moser 2014; Klepzig et al. 2001a; 2001b; Moser 1985; Roets et al. 2009; Theron-de Bruin et al. 2018).

Most knowledge regarding the associations between arthropods, ophiostomatoid fungi, and host trees is confined to the Northern Hemisphere. South African studies have focussed on non-native, economically important forestry trees like *Pinus* L. spp., *Eucalyptus* L'Hér spp. and *Acacia* Mill. spp. (De Beer et al. 1995; Kamgan Nkuekam et al. 2012a; Zhou et al. 2001, 2006). However, there has been increased interest in those from native trees (De Beer et al. 1995; Kamgan Nkuekam et al. 2008; Musvuugwa et al. 2015, 2016a; Roets et al. 2006; 2008; Van der Colff et al. 2017) as host jumps between native and exotic trees may prove to be ecologically and economically devastating (Musvuugwa et al. 2016b). In the present study, we set out to build on these initial studies and document the diversity of sub-cortical beetles, mites, and their ophiostomatoid associates on Afromontane trees to provide a platform for future studies on these important symbioses. In this study, we define an association between different taxa as when 1) a beetle emerged from a host tree, 2) a mite was collected from the surface of a beetle that emerged from a host tree, 3) a mite was collected from a beetle gallery or a wound on a tree, and 4) a fungus was isolated either from a beetle gallery, a beetle, a mite or a tree wound. We therefore do not imply any close and long-term interaction between any two taxa (symbiosis) as this would need additional corroborative studies.

MATERIALS AND METHODS

Arthropod sampling

Sampling was conducted in various Afromontane forest patches throughout the Western Cape province between 2010 and 2012 (Table 1). Bark and wood samples were collected only from storm-damaged trees and trees with signs of sub-cortical beetle activity. When present within native forests, samples were also collected from invasive exotic tree species (e.g. *Pinus radiata* D. Don and *Acacia mearnsii* De Wild). Initial collection of beetles was conducted in the field by inspection of infested trees and wood and aseptically placing individual beetles in sterile vials until further processing. Plant material showing signs of beetle activity (as indicated by small holes and/or frass) was placed in insect emergence cages following the methods of Musvuugwa et al. (2015). Emergence cages were maintained at room temperature and inspected for beetles every 2–3 days for 50 days. All emerging beetles were collected, assigned to morpho-species, their numbers recorded and individually stored at 4 °C until further use (but no longer than 5 days). Reference collections of all beetle taxa collected in this study were stored in 70% ethanol.

Collected beetles were inspected for the presence of phoretic mites using a Leica EZ4 dissection microscope (Leica Microsystems, Taiwan) and when present, these were aseptically removed and individually stored in Eppendorf tubes at 4 °C until further use. In addition, mites were collected directly from bark and wood samples and treated similarly. Reference material of all mite taxa collected in this study was permanently fixed on microscope slides following the methods of Theron et al. (2012). All reference material was sent for identification to the lowest taxonomic level by expert mite and beetle taxonomists and is maintained in the Insect Collection of Stellenbosch University (USEC), Stellenbosch, South Africa.

Fungal isolation from plant material

A subset of collected wood and bark samples was examined for ophiostomatoid fungi using a dissection microscope. When no ophiostomatoid fungi were present, collected material was placed in moisture chambers (re-sealable plastic bags with ddH$_2$O-moistened paper towels) at room temperature (~23 °C) in the dark for up to four weeks to stimulate fungal growth. Where present, masses of ascospores and/or conidia were collected from the apices of sporulating structures using a sterile needle and transferred to Petri dishes containing 2% malt extract agar (MEA, Biolab, Midrand, South Africa) and streptomycin sulphate (0.04 g/l) to inhibit the growth of bacteria (Harrington 1981). Isolates were stored in the dark at room temperature and examined daily for fungal growth. Isolates were purified by transferring single hyphal tips from the edges of actively growing fungal colonies to fresh MEA plates.

Fungal isolation from arthropods

Fungi were isolated from sub-cortical beetles and mites following the methods of Musvuugwa et al. (2015). Briefly, between 3 and 50 individuals (depending on availability) were individually placed in Eppendorf tubes containing 0.2 ml ddH$_2$O and vigorously shaken for 1 min on a vortex mixer. The arthropods were then removed from the tubes and stored in 70% ethanol for later identification. Suspensions were plated on Petri dishes containing MEA. When present, a single representative colony of all suspected ophiostomatoid morpho-types growing per

Table 1. Study sites from which samples were collected

Site	GPS Coordinates
Harold Porter National Botanical Garden	34°20′54″ S, 18°55′31″ E
Assegaaibos Nature Reserve	33°58′23″ S, 18°56′11″ E
Kirstenbosch National Botanical Garden	33°59′11″ S, 18°25′34″ E
Knysna	34°00′21″ S, 23°07′00.6″ E
Storms River	33°05′16″ S, 18°25′07″ E
Goudveld forest	33°54′44″ S, 23°0′10″ E
Groenkop forest	33°56′32″ S, 22°32′50″ E
Gouna forest	33°57′3″ S, 23°2′10″ E
Oubos forest	33°34′60″ S, 23°15′0″ E
J.S. Marais Park	33° 55′57″ S, 18° 52′33″ E
Kogelberg Nature Reserve	34°16′25″ S, 18°54′32″ E
Keurboomstrand	34°0′47.24″ S, 23° 24′54″ E
Silvermine Nature Reserve	34°05′28″ S, 18°25′18″ E

primary isolation plate (one plate per arthropod individual) was randomly chosen as representative and purified. Once purified, all cultures were maintained on Petri dishes containing MEA at 4 °C until further use.

The frequency with which a particular fungus morpho-type was isolated from a particular arthropod species was calculated as F = (NS/NTs) × 100; where F represents the frequency of occurrence (%) of the fungus from each niche, NT represents the total number of samples from which isolations were made and NS represents the number of samples from which fungi were isolated (Yamaoka et al. 1997).

Fungal identification

All fungal cultures obtained were grouped according to morpho-type based on cultural and micro-morphological characteristics. Morpho-types originating from different hosts and different arthropod taxa were not cross-referenced and dealt with as discrete units for molecular characterisation. In total, 222 isolates were grouped according to morpho-type, of which 80 were selected for DNA characterisation. Representative cultures of all morpho-types collected in this study are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, South Africa.

For molecular characterisation, three or more isolates representing each fungal morpho-type were randomly chosen. For DNA extraction, fungal mycelium was collected from two-week-old actively growing colonies on MEA using a sterile scalpel. Genomic DNA was extracted using a Sigma-Aldrich™ plant extraction kit (USA) according to the manufacturer's instructions. PCR reaction conditions and methods for DNA amplification of the nuclear ribosomal internal transcribed spacer region (ITS1, ITS2) and the 5.8S gene region of the rDNA (ITS), followed that described by Musvuugwa et al. (2015, 2016a). For ITS, the primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) were used and where amplification was difficult, ITS1F was replaced with ITS1 (White et al. 1990). According to the initial identification of isolates based on ITS data, amplification of other gene regions for specific taxa was chosen based on previous studies (De Beer et al. 2013; Musvuugwa et al. 2015, 2016a). These included amplification of part of the Beta-tubulin (Bt) gene region (using the primers Bt2a and Bt2b (Glass and Donaldson 1995) or Bt2b and T10 (O'Donnell and Cigelnik 1997) when the aforementioned did not work), the ribosomal Large Sub-unit (LSU, using primers LR0R and LR5 (Vilgalys and Hester 1990)) and Small Sub-unit (SSU, using primers NS1 and NS4 (White et al. 1990)), as well as part of the Calmodulin (CAL) gene (using the primers CL2F and CL2R (Duong et al. 2012) and in cases where amplification was difficult, CL2R2 (Duong et al. 2012), was used instead of CL2R.) were amplified following methods outlined in Musvuugwa et al. (2015, 2016a). Amplified PCR products were cleaned using the EXOSAP-IT kit (USB Corporation, Cleveland, Ohio, U.S.A.) following the manufacturer's instructions. Fragments were sequenced using the respective PCR primers and a Big Dye™ Terminator v3.0 cycle sequencing premix kit (Applied Biosystems, Foster City, CA, U.S.A.) and analysed on an ABI PRISIM™ 3100 Genetic Analyser (Applied Biosystems, Foster City, CA, U.S.A.). The same primers used for PCR amplifications were used and both DNA strands were sequenced. The CLC Genomics Workbench software package (CLC Bio, Cambridge, MA) was used to edit and construct consensus sequences.

Identification of the isolates was done by performing BLAST (Basic Local Alignment Search Tool) searches to compare sequences generated in the present study to sequences previously published on the GenBank sequence database (http://www.ncbi.nlm.nih.gov).

RESULTS

Sub-cortical beetles and their associated mites

When including data from previous studies (Musvuugwa et al. 2015), more than 4500 individuals belonging to at least 14 different genera of sub-cortical beetles (all within the family Curculionidae) were collected from 13 native hosts and the exotic *Pinus radiata* (Table 2). *Lanurgus xylographus* from *Olea capensis* ssp. *macrocarpa* and *Hapalogenius fuscipennis* from *Virgilia oroboides* were abundant, with more than 2000 individuals often emerging from single collections of wood. In other cases, only a single individual of a beetle species was collected, as was the case for *Amphiscolytus capensis* from *Rhoicissus tomentosa*. Several beetle species were collected from more than one host, while a few (e.g. *Cryphalus* sp. 2 and Platypodinae sp. 1) were only collected from a single host (Table 2). Three beetle species were only collected from *Pinus radiata*. Several host trees (e.g. *R. melanophloeos*, *O. capensis* ssp. *macrocarpa*, *Maytenus acuminata*, *V. oroboides* and *Gonioma kamassi*) were associated with more than one beetle species.

Only a few beetle species were associated with phoretic mites (Table 2). Individuals of *L. xylographus* from *O. capensis* ssp. *macrocarpa* were associated with *Dendrolaelaps quadrisetus* and *Elattoma* sp. 1. *Cryphalus* sp. 1 and *H. fuscipennis* were associated with the same *Elattoma* mite, while the pine-associated beetle *Orthotomicus erosus* was associated with *Elattoma* sp. 2, *Histiogaster* sp. 3 as well as *D. quadrisetus* (Table 2).

Mites from wounds on native trees

In addition to phoretic mites, several mites belonging to 18 different morpho species were collected from 12 native tree species (Table 3). *Rapanea melanophloeos* had the highest number of mite species associated with its wounds, with seven mite species collected from this host. A few of the mite species collected were found on more than one host tree species, while others such as Tetranychidae sp. 1, *Uroobovella* sp. 1 and Acaridae sp. 1 seemed to be more specific towards their *R. melanophloeos* host.

Fungi and their host trees

In total, 16 species of ophiostomatoid fungi belonging to six different genera (*Ophiostoma*, *Sporothrix*, *Graphilbum*, *Raffaelea*, *Ceratocystiopsis* and *Graphium*) were collected from 15 different host tree species, including the non-natives *P. radiata* and *Acacia mearnsii* (Table 4). The highest number of ophiostomatoid species was collected from *R. melanophloeos* (Table 4). In addition to fungi collected in previous studies in Afromontane forests (Musvuugwa et al. 2015, 2016a, 2020), six more distinct Operational Taxonomic Units (OTU's) were isolated from diverse hosts in this study. Comparisons with sequences on GenBank confirmed the identity of most of these species but also suggested that some taxa likely represent undescribed species. An unknown fungus, identified as *O. pluriannulatum*-like, was collected from several native hosts, including *R. melanophloeos*, *O. capensis* ssp. *macrocarpa*, *Curtisia dentata*, *Pterocelastrus* sp., as well as from the exotic *A. mearnsii*. An undescribed *Graphium* sp. was collected from *Ilex mitis*. *Ophiostoma quercus* was isolated from a variety of native hosts and from *A. mearnsii* (Table 4). Similarly, *S. fusiforme* was isolated from both a native host (*Brabejum stellatifolium*) and an exotic host (*A. mearnsii*). Three fungal species were exclusively associated with *P. radiata*, namely *O. ips*, *S. pseudoabietina* and an undescribed *Ceratocystiopsis* sp. (Table 4).

Arthropod associated fungi

In total, seven ophiostomatoid fungal species were associated with seven species of sub-cortical beetles. Three of the fungal species were associated with *Pinus radiata* and the rest were

Table 2. Summary of the sub-cortical beetles, host trees and their phoretic mites collected in this study. HPNBG = Harold Porter National Botanical Garden; KNR = Kogelberg Nature Reserve; SNR = Silvermine Nature Reserve. Associations between taxa reported here for the first time (to the best of our knowledge) are indicated in bold.

Beetle species	Reference numbers	Abundance	Host tree	Site	Phoretic mites (reference number)
Amphiscolytus capensis Schedl	T24; T40	2	*Rhoicissus tomentosa* (Lam.) Wild & Drumm	Keurboomstrand	None observed
Cossoninae sp. 1	T2; T3; T11; T29; T31; T37; T38	9	*Scolopia mundii* (Eckl. & Zeyh.) Warb	Oubos Forest	None observed
Cossoninae sp. 2	T5	2	*Virgilia oroboides* (P. J. Bergius) Salter	HPNBG	None observed
Cossoninae sp. 3	T7	1	*Nuxia floribunda* Benth.	Groenkop Forest	None observed
Cryphalus Erichson sp. sp. 1	N2	200	*Virgilia oroboides*	HPNBG	***Elattoma* Mahunka sp. 1** (M6)
Cryphalus Erichson sp. sp. 2	T20	2	Unidentified sp. 2	Goudveld Forest	None observed
Ctonoxylon Hagedorn sp. 1	T8A; T12, T21; T25; T27; T32,	>350	*Gonioma kamassi* (E. Mey.) *Maytenus acuminata* (L.f.) Loes *Olea capensis* ssp. *macrocarpa* L.	Goudveld Forest	None observed
Ctonoxylon Hagedorn sp. 2	T28	>300	*Olea* sp.	Gouna Forest	None observed
Hapalogenius fuscipennis Chapuis	N3	>2000	*Virgilia divaricata* Adamson	Storms River	***Elattoma* sp. 1** (M7)
Hylastes angustatus Herbst	T19	11	*Pinus radiata* D. Don	Knysna	None observed
Hylurgus ligniperda Fabricius	T16	10	*Pinus radiata*	Knysna	None observed
Hypothenemus Westwood sp. 1	T6	2	Unidentified sp. 1	KNR	None observed
Hypothenemus Westwood sp. 2	T14; T15; T18; T27A; T33	27	*Halleria lucida* L *Maytenus acuminata* *Olinia ventosa* L. Cuford *Rapanea melanophloeos* Mez.	HPNBG	None observed
Lanurgus xylographus **Schedl**	T1; T8; T13; T22; T26; T30; T34; T39	>2000	***Gonioma kamassi*** *Olea capensis* ssp. *macrocarpa*	Goudveld Forest	***Dendrolaelaps quadrisetus*** Berlese (M23), ***Elattoma* sp. 1** (M34)
Lanurgus Eggers sp. 2	T23	2	*Rhoicissus tomentosa*	Keurboomstrand	None observed
Corditarsus australis Schedl	N1	700	*Virgilia oroboides*	SNR	None observed
Orthotomicus erosus Wollaston	T17; T35	>100	*Pinus radiata*	J. Marais Park, Knysna	*Dendrolaelaps quadrisetus* (M21), *Elattoma* sp. 2 (M22), *Histiogaster* Berlese sp. 3 (M20)
Platypodinae sp. 1	T43	3	*Rapanea melanophloeos*	Goudveld Forest	None observed
Xyleborinus aemulus Wollaston	T9, T10, T41	16	***Cunonia capensis*** L. ***Rapanea melanophloeos***	Groenkop; HPNBG	None observed
Xyleborinus saxesenii Ratzeburg	T4; T42	117; 5	***Gonioma kamassi*, *Metrosideros angustifolia*** (L.) S.	Assegaaibos; Gouna Forest	None observed

from two native trees hosts *R. melanophloeos* and *O. capensis* ssp. *macrocarpa* (Table 5). Reproductive propagules of *O. ips* were carried by all three of the beetle species (*O. erosus*, *H. ligniperda* and *H. angustatus*) associated with *Pinus radiata*. The undescribed fungal species (*Ceratocystiopsis* sp.) and *S. pseudoabietina* from *Pinus radiata* were also associated with *O. erosus* (Table 5). *Sporothrix pallida* was associated with two beetle species, *L. xylographus* and *Ctonoxylon* sp. 1 from *O. capensis* ssp. *macrocarpa*, while *S. aemulophila* was collected from *X. aemulus* infesting *R. melanophloeos*. *Raffaelea rapaneae* and *R. vaginata* were associated with Platypodinae sp. 1 from *R. melanophloeos* and *L. xylographus* from *O. capensis* ssp. *macrocarpa*, respectively.

Several ophiostomatoid fungal species were associated with mites from 12 different genera (Table 5), including the phoretic *D. quadrisetus*, *Histiogaster* sp. 3, *Elattoma* sp. 1 and *Elattoma* sp. 2. In all cases, the same fungal species as collected from the associated beetle was also isolated from their phoretic mites. For example, *S. pallida* was isolated from both the beetle (*L. xylographus*) associated with *O. capensis* ssp. *macrocarpa* and its phoretic mite *D. quadrisetus*. Interestingly though, three other fungal species from pines (*O. ips*, *Ceratocystiopsis* sp. 1 and *S. pseudoabietina*) were also associated with *D. quadrisetus* and its associated beetle, *O. erosus*. Some of the fungi were associated with numerous mite species (Table 5).

DISCUSSION

This study represents one of only a few on associations between sub-cortical beetles, mites, and ophiostomatoid fungi associated with trees in the Afromontane forests of South Africa. These Afromontane forests are seemingly home to a high diversity of these organisms. Thirteen ophiostomatoid species, 19 mite species and 17 beetle species were collected from only 12 native tree species and some seemed to be specific towards their hosts. Considering that there are more than 90 tree species in these forests, it is reasonable to assume that the diversity of these organisms is still very poorly documented, and many taxa await discovery and formal description (De Beer *et al*. 1995; Kamgan Nkuekam *et al*. 2008; Musvuugwa *et al*. 2015, 2016a; 2020, Roets *et al*. 2006; 2008; Van der Colff *et al*. 2017). The exact nature of the associations between the different taxa still needs to be determined but it will provide valuable information on the ecology of these important fungi.

Four phoretic mite species were collected in this study. Interestingly, in this first report of this mite in South Africa, *D.*

Table 3. Mites associated with wounds on storm-damaged Afromontane forest trees. HPNBG = Harold Porter National Botanical Garden; KNR = Kogelberg Nature Reserve; KNBG = Kirstenbosch National Botanical Garden. Associations between taxa reported here for the first time (to the best of our knowledge) are indicated in bold.

Mite species	Reference number	Host	Site
Acaridae sp. 1	M11; M12	***Rapanea melanophloeos***	Groenkop Forest Goudveld Forest
Acaridae sp. 2	M15	***Rapanea melanophloeos***	HPNBG
Dendrolaelaps Halbert sp. 1	M32	***Trichocladus crinitus*** (Thunb.) Pers	Gouna Forest
Histiogaster Berlese sp. 1	M24	**_Olea capensis_ ssp. _macrocarpa_**	Groenkop Forest
Histiogaster Berlese sp. 2	M30	Unidentified woody climber sp. 2	KNBG
Lasioseius Berlese sp. 1	M19; T31; T33	***Curtisia dentata*** C.A. Sm. **_Olea capensis_ ssp. _macrocarpa_** Unidentified woody climber sp. 1	Gouna Forest KNBG Goudveld Forest
Mesostigmata sp. 1	M13; M18	***Curtisia dentata*** ***Rapanea melanophloeos***	Groenkop Forest Gouna Forest
Oribatida sp. 1	M16; M27	***Trichocladus crinitus*** ***Rapanea melanophloeos*** ***Brabejum stellatifolium*** L	Knysna Gouna Forest Assegaaibosch
Oribatida sp. 2	M17; M28	***Curtisia dentata*** ***Platylophus trifoliatus*** (Lf) D. Don	Gouna Forest Oubos Forest
Paraleius Berlese sp. 1	M1; M8	***Halleria lucida*** ***Rapanea melanophloeos***	Gouna Forest HPNBG
Paraleius Berlese sp. 2	M2	Unidentified sp. 1	KNR
Proctolaelaps Berlese sp. 1	M25; M29	**_Olea capensis_ ssp. _macrocarpa_**	Groenkop Forest Goudveld Forest
Proctolaelaps Berlese sp. 2	M26	***Trichocladus crinitus***	Gouna Forest
Tetranychidae sp. 1	M14	***Rapanea melanophloeos***	KNBG
Trichouropoda Berlese sp. 1	M3; M4	***Podalyria sericea*** (Andrews) R.Br. ex Aiton f. *Virgilia oroboides*	KNBG
Trichouropoda Berlese sp. 2	M10	***Rapanea melanophloeos***	HPNBG
Uroobovella Berlese sp. 1	M9	***Rapanea melanophloeos***	Groenkop Forest
Unidentified sp.	M5	*Virgilia oroboides*	KNBG

quadrisetus was phoretic on two beetle species, *L. xylographus* from native *O. capensis* ssp. *macrocarpa* and *O. erosus* from exotic *P. radiata*. *Orthotomicus erosus* has been collected from *Pinus* spp. in Europe, the Mediterranean region and the Middle East, where it is assumed to be native (Ferreira & Ferreira 1986). *Dendrolaelaps quadrisetus* may therefore be of European and North American origin where it is known to associate with pine-infesting bark beetles such as *Dendroctonus rufipennis* (Cardoza *et al.* 2008; Fernandez *et al.* 2013; Gwiazdowicz *et al.* 2012). *Orthotomicus erosus* is exotic to southern Africa and this may also be the case for *D. quadrisetus*. When arthropods such as conifer-infesting bark beetles are introduced into new habitats, they also introduce their associated fungi and phoretic mites (Bridges & Moser 1983). *Dendrolaelaps quadrisetus* was seemingly able to move from its natural associates (*Pinus* spp. and *O. erosus*) to *L. xylographus* which is only known from native trees. *Dendrolaelaps quadrisetus* is a predator that feeds on eggs and larvae of its phoront beetle and nematodes (Kinn 1967). It is possible that the eggs and larvae of *L. xylographus* might be palatable to *D. quadrisetus* or that the beetle carries nematodes that are palatable to the mite, which could have facilitated the possible host jump from *O. erosus*.

While it is known that organisms associated with introduced bark beetles can form associations with newly encountered beetles and hosts, this phenomenon is only known for closely related hosts unlike the situation identified here (Jacobs *et al.* 2004; Wingfield *et al.* 2013). Host jumps by these organisms could have serious ecological and economic consequences, especially if pathogenic fungi are transferred to newly encountered hosts. For example, of the several ophiostomatoid fungal species tested for their pathogenicity on six different host trees, at least two fungal species were pathogenic on four tree species (Musvuugwa *et al.* 2016b).

Despite a wealth of studies on ophiostomatoid fungi in plantation forestry in South Africa, we identified undescribed taxa in our study. An undescribed *Ceratocystiopsis* species was collected from *P. radiata* that was associated with the well-known forestry pest *O. erosus* and its phoretic mite *D. quadrisetus*. The origin of this fungal taxon remains uncertain, but it may have been introduced from outside of South Africa. Most of the members of this genus are only known as pine associates (Hsiau & Harrington 1997; Zhou *et al.* 2001) and no *Pinus* species are native to South Africa. Conversely, it is also possible that this *Ceratocystiopsis* may be a native species that broadened its host range to exotic *Pine* species (Nel et al. 2021). This uncertainty highlights the need for continued monitoring to identify and track introductions of exotic organisms into South Africa, and native species to countries outside of South Africa, as these may have undesirable future consequences (Musvuugwa *et al.* 2016b).

Ophiostma quercus and the *O. pluriannulatum*-like fungus were collected from several native hosts and the exotic *A. mearnsii*, while *S. fusiforme* was isolated from *B. stellatifolium* and *A. mearnsii*. *Ophiostoma quercus* is a well-known fungus in both the Southern and Northern Hemisphere and is known to colonise many hardwood species (De Beer *et al.* 2003; Harrington *et al.* 2001). It was therefore not surprising to collect it from several hosts here. It has previously also been isolated from *Terminalia sericea* (Roxb.) Wight & Arn., *Olinia* sp. Thunberg. *Quercus robur* L., *V. oroboides* and the exotic *Eucalyptus grandis* W. Hill ex Maiden in South Africa (De Beer *et al.* 1995; Kamgan Nkuekam *et al.* 2008). Similarly, *O. pluriannulatum* was previously collected from several hardwood and conifer hosts in North America (Appel *et al.* 1990), Europe (Romón *et al.* 2007) and South Africa (Zhou *et al.* 2006, Net et. al. 2021).

Sporothrix fusiforme was first described from *Populus nigra* L., *Castanea sativa* Mill., *Q. petraea* (Mattuschka) Liebl and *Larix decidua* Mill from Azerbaijan and Austria (Aghayeva *et al.* 2004). Increased globalisation of trade in plants, as well as flaws in the

Table 4. Ophiostomatoid fungi associated with different host trees in Afromontane forests, their culture collection (CMW) and GenBank accession numbers, their closest matching species in GenBank, matching % and gap %. HPNBG = Harold Porter National Botanical Garden; KNR = Kogelberg Nature Reserve; KNBG = Kirstenbosch National Botanical Garden**.** Associations between taxa reported here for the first time (to the best of our knowledge) are indicated in bold.

Fungal species	CMW number	ITS	BT	CAL	LSU	Closest matching species (GenBank number) or reference	Matching %	Gaps %	Host plant	Site
Ceratocystiopsis Upadhyay & Kendrick sp.1	CMW 40346 CMW 40347 CMW 40348		PQ201090			*Ceratocystiopsis* sp. Bt, (MW066349)	100	0/555 (0)	*Pinus* sp.	Knysna
Graphilbum roseum Musvuugwa, Dreyer & Roets	CMW 40349 CMW 40350 CMW 40351 CMW 40353	KY050751 KY050753 KY050752 KY050754				Musvuugwa **et al.** 2020			*Curtisia dentata* *Halleria lucida* *Rapanea melanophloeos*	Gouna Forest Gouna Forest Groenkop Forest
Graphium Corda sp. 1	CMW 40354 CMW 40355 CMW 40356	KY050757 KY050755 KY050756				*Graphium euwallaceae*, EF, (KF534799)	95	2/644 (0%)	***Ilex mitis*** (L.) Radik	Assegaaibosch
Ophiostoma ips (Rumbold) Nannf.	CMW 40322	KY050748	KY094081			*Ophiostoma ips*, Bt, (FJ012142)	99	1/460 (0%)	*Pinus radiata*	Knysna
Ophiostoma pluriannulatum (Hedgc.) Syd**. -lik**e	CMW 40333 CMW 40334 CMW 40335 CMW 40336		PQ201092			*Ophiostoma sparsinnulatum*, Bt, (FJ907176)	95	2/256 (0%)	***Olea capensis*** ***Rapanea melanophloeos*** ***Pterocelastrus*** Meisn sp. ***Curtisia dentata***	Storms River Goudveld Forest, Gourna Forest Groenkop Forest
Ophiostoma quercus (Georgévitch) Nannf	CMW 40337 CMW 40341 CMW 40345	KY050747 KY050746 KY050745	KY094082 KY094083 KY094084			*Ophiostoma quercus*, Bt, (HM041856)	100	0/211 (0%)	***Trichocladus crinitus*** ***Rapanea melanophloeos*** ***Olea capensis*** ***Curtisia dentata*** *Acacia mearnsii*	Gouna Forest Groenkop Forest Goudveld Forest Knysna
Ophiostoma stenoceras (Robak) Nannf.	CMW 40372 CMW 40373 CMW 40374	KU595580 KU595579	KU639619 KU639620 KU639612	KU639613 KU639614 KU639615		*Ophiostoma stenoceras*, Bt, (AY280472)	100	0/290 (0%)	***Rapanea melanophloeos*** ***Virgilia oroboides***	KNBG
Sporothrix oleae Musvuugwa, Dreyer & Roets	CMW 40360 CMW 40361 CMW 40362	KY050758 MN298850 MN298851	KY094078 KY094079 KY094080			Musvuugwa **et al.** 2020			*Olea capensis*	Goudveld Forest
Sporothrix pseudoabietina Wang, Lu & Zhang	CMW 40378 CMW 40379 CMW 40380		PQ201091			*Sporothrix pseudoabietina*., Bt, (MW066385)	100	0/473 (0%)	*Pinus radiata*	JS Marais Park
Sporothrix aemulophila Musvuugwa, Dreyer & Roets	CMW 40381 CMW 40382 CMW 40383	KT192603	KT192607 KT192608 KT192609	KT192613		Musvuugwa **et al.** 2015			*Rapanea melanophloeos*	HPNBG
Sporothrix fusiforme Aghayeva & Wingf.	CMW 40319 CMW 40320 CMW 40321	KY050749 KY050750	KY094085 KY094086 KY094087			*Sporothrix fusiforme*, Bt, (AY280464)	100	0/286 (0%)	***Acacia mearnsii*** ***Brabejum stellatifolium***	Assegaaibosch
Sporothrix itsvo Musvuugwa, Dreyer & Roets	CMW 40370 CMW 47169 CMW 40371	KU595582	KU639625 KU639626 KU639628			Musvuugwa **et al.** 2016a			*Rapanea melanophloeos*	HPNBG
Sporothrix pallida (Tubaki) Matsushima	CMW 40330 CMW 40331 CMW 40332	KT192606 KT192605 KT192604	KT192610 KT192611 KT192612	NA NA KT192614		*Sporothrix pallida*, Bt, (EF139110)	100	0/301 (0%)	*Olea capensis*	Goudeveld Forest
Sporothrix rapaneae Musvuugwa, Dreyer & Roets	CMW 40367 CMW 40368 CMW 40369	KU595581 KU595583	KU639622 KU639623 KU639624	KU639608 KU639609		Musvuugwa **et al.** 2016a			*Rapanea melanophloeos*	Groenkop Forest
Raffaelea rapaneae Musvuugwa, Dreyer & Roets	CMW 40358 CMW 40359 CMW 40357	KT192597 KT192601 KT192596			KT182934 KT182935 KT182930	Musvuugwa **et al.** 2015			*Rapanea melanophloeos*	Gourna forest
Raffaelea vaginata Musvuugwa, Dreyer & Roets	CMW 40363 CMW 40364 CMW 40365 CMW 40366	KT192598 KT192599 KT192602 KT192600			KT182931 KT182932 KT182933	Musvuugwa **et al.** 2015			*Olea capensis*	Gouldveld Forest

Table 5. Ophiostomatoid fungi associated with different host trees in Afromontane forests, their isolation frequency (F), and association with sub-cortical beetles and mites. HPNBG = Harold Porter National Botanical Garden; KNR = Kogelberg Nature Reserve; KNBG = Kirstenbosch National Botanical Garden. Associations between taxa reported here for the first time (to the best of our knowledge) are indicated in bold.

Fungal species	Host plant	Arthropod species (B = beetle, M = mite)	*N*	F (%)	Site
Ceratocystiopsis Upadhyay & Kendrick sp.1	*Pinus radiata*	***Dendrolaelaps quadrisetus*** (M)	20	10	Kysna
		Orthotomicus erosus (B)	50	14	
Graphilbum roseum Musvuugwa, Dreyer & Roets	*Curtisia dentata*	**Oribatida sp. 2** (M)	50	38	Gouna Forest
	Curtisia dentata	***Lasioseius* sp. 1** (M)	12	28	
	Curtisia dentata	**Mesostigmata sp. 1** (M)	6	50	
	Halleria lucida				Gouna Forest
	Rapanea melanophloeos				Groenkop Forest
Graphium Corda sp. 1	***Ilex mitis*** (L.) Radik				Assegaaibosch
Ophiostoma ips (Rumbold) Nannf.	*Pinus radiata*	*Orthotomicus erosus* (B)	50	58	Knysna
		Hylurgus ligniperda (B)	10	40	
		Hylastes angustatus (B)	11	45	
		Dendrolaelaps quadrisetus (M)	20	25	
		***Histiogaster* sp. 3** (M)	15	6	
		***Elattoma* sp. 2** (M	30	6	
Ophiostoma pluriannulatum (Hedgc.) Syd.**-like**	***Olea capensis***	**Acaridae sp. 1** (M)	15	33	Storms River
	Rapanea melanophloeos				Goudveld Forest,
	Olea capensis	***Mesostigmata* sp. 1** (M)	8	25	Gourna Forest
	***Pterocelastrus* Meisn sp.**				Goudveld Forest
	Curtisia dentata				Gouna Forest
	Rapanea melanophloeos	***Uroobovella* sp. 1** (M)	15	20	Gouna Forest
	Acacia mearnsii				Groenkop Forest
	Rapanea melanophloeos				Knysna
Ophiostoma quercus (Georgévitch) Nannf	***Trichocladus crinitus***	**Oribatida sp. 1** (M)	30	30	Gouna Forest
	Rapanea melanophloeos	***Dendrolaelaps* sp. 1** (M)	9	56	Groenkop Forest
	Olea capensis	**Oribatida sp. 2** (M)	50	44	Goudveld Forest
	Curtisia dentata	***Proctolaelaps* sp. 2** (M)	17	29	Gouna Forest
	Acacia mearnsii				Knysna
Ophiostoma stenoceras (Robak) Nannf.	***Rapanea melanophloeos***				KNBG
	Virgilia oroboides				
Sporothrix oleae Musvuugwa, Dreyer & Roets	*Olea capensis*				Goudveld Forest
Sporothrix pseudoabietina Wang, Lu & Zhang	*Pinus radiata*	***Orthotomicus erosus*** (B)	50	16	JS Marais Park
		Dendrolaelaps quadrisetus (M)	20	15	
Sporothrix aemulophila Musvuugwa, Dreyer & Roets	*Rapanea melanophloeos*	*Xyleborinus aemulus* (B)	6	67	HPNBG
		***Paraleius* sp. 1** (M)	12	17	
		***Trichouropoda* sp. 2** (M)	50	34	
Sporothrix fusiforme Aghayeva & Wingf.	***Acacia mearnsii***	**Oribatida sp. 1** (M)	19	11	Assegaaibosch
	Brabejum stellatifolium				
Sporothrix itsvo Musvuugwa, Dreyer & Roets	*Rapanea melanophloeos*				HPNBG
Sporothrix pallida (Tubaki) Matsushima	*Olea capensis*	***Cytonoxylon*** sp. 1 (B)	25	60	Goudveld Forest
		Lanurgus xylographus (B)	50	68	
		Dendrolaelaps quadrisetus (M)	50	64	
		***Elattoma* sp. 1** (M)	20	45	
Sporothrix rapaneae Musvuugwa, Dreyer & Roets	*Rapanea melanophloeos*				Groenkop Forest
Raffaelea rapaneae Musvuugwa, Dreyer & Roets	*Rapanea melanophloeos*	Platypodinae sp. 1 (B)	1	100	Gouna forest
Raffaelea vaginata Musvuugwa, Dreyer & Roets	*Olea capensis*	*Lanurgus xylographus*	50	59	Goudveld Forest

international protocols for plant biosecurity, has led to increased events of organisms utilising newly encountered hosts (Brasier & Webber 2010) and may have led to the introduction of this fungus in South Africa. It is not known whether *S. fusiforme* is pathogenic towards its hosts in its native ranges. However, its movement native hosts in South Africa may be problematic, even if it is not pathogenic to these newly encountered hosts, as it may be able to outcompete native fungi and disrupt normal ecosystem processes.

Although most of the fungal species collected from native hosts were only specific to one native host, a couple of wound-associated fungal species were found on a large variety of native hosts. These were *S. pallida*, *G. rosea*, *O. quercus,* and the *O. pluriannulatum*-like fungus. Interestingly, these species were also associated with several mite species. Associations with numerous mites may aid successful transport between different host species as more dispersal agents would have a greater variety in mobility and niche requirements.

Ophiostoma pluriannulatum has previously been isolated from several wood- and bark-infesting insects, including bark beetles and nitidulids (Appel *et al*. 1990; 2006; Romón *et al*. 2007; Zhou *et al*. 2004). It has also been isolated from a Bostrichidae beetle in South Africa (Nel et al. 2021) and may therefore be a common associate of tree-associated arthropods. Like *G. roseum, O. pluriannulatum*-like is also pathogenic to native and introduced hosts in South Africa including *R. melanophloeos* and *A. mearnsii* (Musvuugwa *et al.* 2016b). The pathogenic nature of these wound-infecting fungal species and the apparent ease of transport between different tree species indicate that they may become significant future threats to forestry, agriculture and/or ornamental tree growing.

To conclude, this study reports multiple previously unknown associations between a large diversity of sub-cortical beetles, ophiostomatoid fungi, and mites from trees in only a few Afromontane forest patches. Some taxa were specific to hosts and/or vectors. However, several taxa are associated with numerous taxa (and hosts in particular) including native and exotic species. Many ophiostomatoid fungi are virulent pathogens to their hosts and rely on their associated organisms for dispersal. This study therefore highlights the need for future studies to unravel the nature of these associations so that potential ecological interferences from exotic organisms can be identified and managed.

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AUTHOR CONTRIBUTIONS

TM: Conceptualization, Data Curation, Methodology, Writing – Original Draft, Writing – Review and Editing.
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