Ophiostomatoid fungi associated with fungus farming insects in South Africa

By

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DECLARATION

I, the undersigned, hereby declare that the thesis submitted herewith for the degree *Philosophiae Doctor* to the University of Pretoria, contains my own independent work and has not been submitted for any degree at this or any other university.

Miss Wilma J. Nel

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To those I have lost,

To those who have come and gone,

And to those who have been with me all the way

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PREFACE

Symbiosis is the term used to describe the different forms of communal life that can exist between two unlike organisms. Primarily this involves interaction in one of three forms: mutualism where both partners benefit from their association; antagonism where one or both partners are harmed by their association; and commensalism where one partner derives benefit but the other is neither harmed nor profited from their association. Many different forms of symbioses have been described between insects and microbes and these include short, simple interactions as well as obligate associations.

Aside from humans, sophisticated agricultural farming practices have only been found for three insect groups, colloquially known as fungus-farmers. These three groups – the attine ants, the macrotermitinae termites, and the ambrosia beetles - each independently evolved an obligate mutualism with fungal partners that they actively cultivate and maintain within their nests and utilize as a primary source of nutrition. The primary focus of this PhD thesis was that of the association that exists between bark and ambrosia beetles and their respective fungal partners and the genomic signatures of these associations. Additionally, the unexpected and interesting discovery of Ophiostomatoid fungi from fungus-growing macrotermitinae termites was also investigated.

Very little research on ambrosia beetles and their fungal partners has been conducted in South Africa. However, the accidental introduction of the devastating Polyphagous Shot Hole Borer into the country has sparked renewed interest into this topic. The primary focus of the research included in this thesis has consequently been on ambrosia beetles in South Africa. This is also the topic of the literature review (**Chapter 1**) at the start of the thesis. However, given the many commonalities regarding their associations with fungi, we have also included a study that led to the discovery of Ophiostomatoid fungi associated with fungus-farming termites. The background literature relating to this association is provided in the introduction to that study and it is also a topic that has been thoroughly treated in a number of recent reviews.

In **Chapters 2-4** I explore the diversity of Ophiostomatoid fungi associated with fungus-farming insects in South Africa. In **Chapter 2**, I report four species of Ophiostomatalean species associated with commonly found ambrosia beetles in the country. One of these species is reported from South Africa for the first time and two are described as novel species. In **Chapter 3**, I report for the first time the presence of the granulate ambrosia beetle and its Microascalean associate, *Ambrosiella roeperi*, in South Africa and highlight the potential threat that this beetle and its fungal symbiont poses to South Africa's agricultural industry. In

Chapter 4, I investigate three species of Ophiostomatalean fungi discovered on the abandoned *Termitomyces* fungus combs of fungus-farming termites. Using both culture- based and genomics techniques, I describe these newly discovered Ophiostomatalean fungi and investigate their distinct lifestyle.

In **Chapter 5,** I delve more deeply into the relationship shared between Sordariomycete fungi known to be associated with arthropods. In this chapter, I attempt to elucidate how the relationship with their arthropod partners has influenced their genomic evolution. Using whole genome sequences and comparative genomics methods I investigate the traits shared amongst the Ascomycete ambrosia fungi, as well as investigate how these fungi differ genetically from their close relatives.

Finally, as a **supplementary chapter** to this thesis, I provide a comprehensive record of bark and ambrosia beetle species present in South Africa. This is justified by the fact that other studies included in this thesis involved extensive trapping of bark and ambrosia beetles, many of which had not previously been recorded in the country. This record increases the currently recorded number of these insects from South Africa from 163 to 260. Additionally, 24 species are reported from the county for the first time. It was decided to place this chapter as supplementary material and not within the main body of the thesis because it did directly capture the common theme of Ophiostomatoid fungi associated with fungus-farming insects.

CHAPTER 1

Literature review:

Ambrosia beetle fungiculture: A South African perspective

Ambrosia beetle fungiculture: A South African perspective

1. Abstract

Symbiosis is used to define three primary forms of interactions that can exist between unlike organisms i.e. mutualism, antagonism, or commensalism. In nature, various symbioses exist between insects and microbes, the most sophisticated of which is probably insect fungiculture, also known as fungus-farming. Only three groups of insects – the attine ants, macrotermitinae termites, and ambrosia beetles – are known to exhibit this sophisticated fungus cultivating behavior. Although various investigations focussed on each of these three interactions have been conducted, the relationship between the ambrosia beetles and their fungal partners remain the least well understood. Therefore, we focus our attention on the ambrosia symbiosis and discuss recent advances in our understanding of how the symbiosis evolved and how it is maintained and explore recent research that contradict some of the early hypotheses surrounding the symbiosis. We also consider the effects that the movement of these insects are having, what makes them successful invaders and provide an overview of the problem being faced in South Africa due to the Polyphagous Shot Hole Borer introduction.

2. Introduction

The term symbiosis is familiar to every biologist where it is used to describe the interactions between organisms based on the definition provided by Anton De Bary (De Bary 1879). Even though De Bary is often credited for introducing the term, it was actually introduced a year earlier by the German botanist Albert Frank (Frank 1877, Klepzig et al. 2009, Martin & Schwab 2012). However, both their reports provided vague and deceptively simple definitions for symbiosis, suggesting only it referred to an interaction between two unrelated species living together.

A contemporary view of symbiosis is one encompassing significantly more than the “living together of two unlike organisms”. And it is for this reason that De Bary’s definition is often favoured over that of Frank. Unlike Frank, De Bary defined three different forms of symbioses, i.e. mutualism, commensalism, and parasitism (often referred to as antagonism), that applied to all organisms that exist in nature. In contrast, Frank’s view was based on specific reference to lichens. Yet, even today, the

definitions describing these interactions are widely debated and open to interpretation and can include a range of associations from brief encounters between different organisms to those that are obligate (Martin & Schwab 2012, 2013).

From its simplest definition, a mutualism can be interpreted as an interaction where two organisms derive benefit from their association (Boucher et al. 1982). Among the insects, there are many different forms of mutualism, ranging from a simple pollinator visiting a flower to the complex agricultural practices of fungus-growing termites. Aside from human agricultural practices, insects are the only other animals known to cultivate and maintain their own crops (Mueller & Gerardo 2002, Mueller et al. 2005). And in this regard, insect fungiculture is likely the most sophisticated mutualism thus-far discovered in nature.

There are three insect groups that are regarded as the true "fungus farmers". These include the new world attine ants (Mehdiabadi & Schultz 2010); the old world Macrotermitinae termites (Aanen et al. 2002); and the wood boring ambrosia beetles (Farrell et al. 2001). The recognition of the importance of ant, termite and ambrosia mutualisms (Klepzig et al. 2009, Bracewell & Six 2015, Hulcr & Stelinski 2017) and the interesting drivers behind these processes, has catalysed new and innovative research on them. However, the relationships between ambrosia beetles and their fungal symbionts remains the least understood of the three systems. This is likely due to the difficulty in studying these insects that arises from their complex lifestyles within wood.

The fact that ambrosia beetles are not considered primary pests in their native environments has implied a reduced interest in studying them (Hulcr et al. 2017). However, in recent years, some invasive ambrosia beetle species have become major economic pests in their introduced ranges (Ploetz et al. 2013, Hulcr et al. 2017), driving a greater interest to this group. Consequently, this review is focussed primarily on ambrosia beetles and their closely related bark beetle relatives. A key objective is to highlight older and recent research on their symbioses with fungi and to consider their relevance in South Africa in the aftermath of the introduction of the devastating ambrosia beetle *Euwallacea formicatus* (commonly known as the Polyphagous Shot Hole Borer) into the country.

3. The evolution of agriculture in insects

Overall fungiculture of ants and termites is very different, but they do share at least a couple of commonalities. One of these is that the symbioses with their fungal partners evolved only once in their histories before diversifying (Aanen et al. 2002, Mehdiabadi & Schultz 2010). In the case of ambrosia beetles, recent studies suggest that fungiculture could have arisen independently as many as 15 times (Hulcr & Stelinski 2017, Vanderpool et al. 2018), although the exact number of times this behaviour has evolved remains to be determined (Hulcr & Stelinski 2017).

In all three these insect groups that live in symbiosis with fungi, the fungal symbionts are vitally important, serving as the primary food source of the growing colonies (Mueller & Gerardo 2002, Mueller et al. 2005, Nobre et al. 2010). Both the ants and the termites cultivate their fungal monocultures as specialized structures deep within their subterranean nests, providing plant debris on which the fungi feed (Aanen et al. 2002, Mueller & Gerardo 2002, Nobre et al. 2011a). However, this is mostly where the similarities between the ant and termite symbioses end. In contrast, the beetles inoculate their symbionts, which they carry along with them in specialized pouches in their exoskeletons known as mycangia, on the walls of their tunnels systems (also known as galleries) that they bore deep into their woody hosts (Farrell et al. 2001, Mueller et al. 2005).

Aside from having the most number of symbiotic origins, fungiculture in ambrosia beetles is also the most diverse and oldest of the three groups, having arisen the first time in the Platypodinae an estimated ~119-88 million years ago (Jordal & Cognato 2012, Jordal 2015, Vanderpool et al. 2018). The diversity of fungiculture in this group however is not surprising with around 4300 described ambrosia beetle species, more than 10 times that of fungus farming termites and ants. The symbioses between the ants, termites and their fungi are estimated to be much younger, having arisen ~55-65 million years ago in the ants (Bransetter et al. 2017) and only ~ 31 million years ago in the termites (Aanen et al. 2002, Nobre et al. 2011b).

In the case of ambrosia beetles, fungiculture has been proposed to have evolved following the “transmission first” model (Mueller & Gerardo 2002, Mueller et al. 2005, Six 2012, Vanderpool et al. 2018). For the termites, evolution of fungiculture is proposed to have evolved following a “consumption

first" model (Mueller et al. 2001, 2005). In the case of the transmission first model, it is proposed that the insects initially acted as vectors of their future symbiont, and that over time, their close association resulted in an obligate relationship. In the consumption first model, it is proposed that an insect began to naturally incorporate a new substrate into its normal diet. Thereafter, and over the course of a long period, the insect emerged as a specialist feeder on the substrate, evolving an obligate relationship (Mueller et al. 2001). However, in the case of the attine ants, it remains unclear which of the two models best fit the mechanism that led to their evolution of fungiculture (Mueller et al. 2001, 2005, Mueller & Gerardo 2002).

4. Differences between ambrosia beetles and their bark beetle relatives

Collectively the Scolytinae and Platypodinae include approximately 7500 described species or roughly 10% of the known diversity of the true weevil family Curculionidae (Mueller et al. 2005, Jordal & Cognato 2012). Of these, almost all (approximately 1350) species of Platypodinae and approximately 3000 species of Scolytinae are ambrosia beetles. The remaining Scolytinae are bark, seed and pith feeding beetles (Farrell et al. 2001, Mueller et al. 2005, Jordal & Cognato 2012, Six 2012). Because these beetles reside in two sub-families of the Curculionidae, the terms bark beetle or ambrosia beetle are not used to distinguish between their phylogenetic relationships, but rather to describe their unique lifestyles (Mueller et al. 2005, Six 2012, Hulcr & Stelinski 2017). Generally, bark and ambrosia beetles are very similar in both their morphology (**Figure 1**) (Cognato et al. 2011) and ecology and the major distinguishing factors between these two groups are in their feeding habits and utilization of plant substrate (Jordal & Cognato 2012, Six 2012, 2019).

The bark beetles represent a polyphyletic assemblage of some 3700 species that are primarily phloem and cambium feeders, some of which supplement their diets by feeding on symbiotic fungi (Haanstad & Norris 1985, Klepzig & Six 2004, Harrington 2005, Massoumi-Alamouti et al. 2009, Six 2012, Hulcr & Stelinski 2017 Vanderpool et al. 2018). In contrast, ambrosia beetles rarely feed on wood and are obligately dependent on their fungal symbionts as a source of nutrition (Cassar & Blackwell 1996, Mueller & Gerardo 2002, Jordal & Cognato 2012, Six 2012, Vanderpool et al. 2018). This also reflects the fact that the word "ambrosia", referring to "the food of Gods" (Hulcr & Stelinski 2017), was first

used in 1836 by Schmidberger referring to the substrate on which the beetles were feeding, rather than the insects themselves (Hubbard 1896, Haanstad & Norris 1985, Hulcr & Stelinski 2017). At the time of its first application to the beetles, it was not known that the white substrate on which the they fed was fungal tissue (Harrington 2005, Hulcr & Stelinski 2017). It was eight years later that this white substrate was recognized as a fungus purposefully cultivated by the beetles along their gallery walls (Hartig 1844, Hubbard 1896, Haanstad & Norris 1985).

The differences in feeding habits of bark and ambrosia beetles are also reflected in the difference in utilization of host substrate by the two groups (**Figure 2**). Bark beetles construct their galleries directly under the bark in the cork cambium (Haanstad & Norris 1985, Harrington 2005, Six 2012). This allows them direct access to the phloem that provides the beetles with most of the nutrients they require (Farrell et al. 2001, Klepzig & Six 2004, Harrington 2005, Jordal & Cognato 2012, Six 2012). In contrast, ambrosia beetles construct their galleries deep within the sapwood of their hosts (Haanstad & Norris 1985). This area of the wood is poor in nutrients and its major component is lignocellulose, a substrate difficult to digest for most insects (Haanstad & Norris 1985, Harrington 2005, Six 2019). The ambrosia beetles consequently rely on their symbiotic fungal associates to act as an external stomach that assists in the concentration of nutrients from the host into nitrogen rich substrates available to the beetles (Kolařík & Kirkendall 2010, Licht & Biedermann 2012, Kasson et al. 2016).

Aside from their feeding habits, ambrosia beetles have a unique biology as well as specialized mating strategies. The galleries of ambrosia beetles are constructed deep within the wood, commonly by a single mated female to lay her eggs (Harrington 2005, Mueller et al. 2005). Once the offspring emerge, the entire brood lives alongside one another, feeding on the ambrosia “fungal garden” propagated by their mother (Mueller et al. 2005). Due to the close quarters within the gallery system, ambrosia beetles display high levels of inbreeding and sib-mating (Jordal et al. 2000, Peer & Taborsky 2005, Marini et al. 2011, Kasson et al. 2013). Consequently, many ambrosia beetles also display haplodiploidy. This sex determination system results in a population of haploid males and diploid females, developing from unfertilized and fertilized eggs respectively (Jordal et al. 2000, Cognato et al. 2011). Haplodiploidy is common in many close community insect groups such as the Hymenoptera (bees, wasps and ants) and Thysanoptera (thrips) and often results in a sex ratio skewed towards females (Peer & Taborsky 2005,

Kasson et al. 2013, Stouthamer et al. 2017). This skew in sex ratio often results in females being the dominant sex responsible for brood establishment and care and the males are commonly small, flightless and rarely venture outside (Jordal et al. 2000, Mueller et al. 2005, Cognato et al. 2011). However, this is not true for all ambrosia beetle species, as seen in species of *Gnathotichus* where the males are the dominant sex responsible for gallery establishment and brood care and the females lack mycangia and the ability to vector fungi (Farris 1962).

5. Evolution of a single structure ensures a stable symbiosis

The majority of ambrosia beetles have evolved a special structure to transport their symbionts (Batra 1963). Pouches in the exoskeleton, known as mycangia, harbor the fungal spores of the symbiont as the beetles move between hosts (Batra 1963, Batra 1966). The mycangia can be present in only a single sex of a species or both sexes, often depending on their respective roles in brood establishment and care (Batra 1963). Although mycangia can present in many forms and complexities, those that adhere to the strict definition of being invaginations lined with secretory gland cells that produce nutrients to aid in the maintenance and growth of the fungal symbiont, have been divided into five classes depending on their location on the beetle body. These classes are prothoracic pleural; pro-mesonatal; elytral; prosternal-subcoxal; and oral (Beaver 1986, Hulcr & Cognato 2010, Hulcr & Stelinski 2017). Although the majority of ambrosia beetle species have mycangia that adhere to this strict definition, some species have simpler structures such as those found in bark beetles resembling pits, sacs or setal brushes that serve as a consistent means to transport fungi (Beaver 1986, Six 2003, Klepzig & Six 2004, Ploetz et al. 2013).

Irrespective of their sophistication, in all cases mycangia of ambrosia beetles serve to maintain the association of these insects and their symbionts. This is similar to attine ant fungal farmers (Mehdiabadi & Schultz 2010) as most ambrosia beetles utilize vertical transmission from the parental galleries to obtain their symbiotic fungi (Ploetz et al. 2013, Carrillo et al. 2014). This means that when a new adult beetle prepares to leave its brood, it picks up spores of the fungal symbiont lining the gallery walls and transfers these to the mycangium before emergence. Once this adult beetle reaches a suitable host and

begins gallery construction, the spores are deposited from the mycangium to the gallery walls, utilizing the same fungus it was reared on to rear its own brood (Beaver 1986).

6. "One size fits all" does not apply to all ambrosia symbioses

Contemporary research is revealing many unexpected and previously unknown habits of ambrosia beetles. These include many important new discoveries, such as the mode by which some species acquire their symbionts (Huclr & Cognato 2010) and how mycangial shape influences symbiont acquisition (Mayers et al. 2018, 2020). Additionally, it challenges the one beetle-one fungus assumption that has been in place since the late 1800s (Kostovcik et al. 2015).

Most ambrosia beetles acquire their symbionts by vertical transmission, filling their mycangia with the fungus cultivated by their mother in the natal gallery. However, some ambrosia beetle species have now been discovered that obtain their symbionts laterally from other beetle species (Hulcr & Cognato 2010, Ploetz et al. 2013, Carrillo et al. 2014, Hulcr & Stelinski 2017). The importance of the mycangium in vertical transmission can be observed in these species, as some species that acquire their symbionts laterally appear to have subsequently lost their mycangia (Hulcr & Cognato 2010, Skelton et al. 2019a). One of the best examples of this situation is found in the mycocleptic (crop stealing) ambrosia beetles. Mycocleptic ambrosia beetles construct their galleries within millimetres of a host beetle gallery, allowing the symbiont of that species to grow into and cultivate their own. Because these beetles no longer need to vector their own symbionts, they have apparently subsequently lost their mycangia. However, this has apparently also caused them to become completely dependent on a host ambrosia beetle gallery (Huclr & Cognato 2010). Exactly how such host galleries are selected remains unclear, but fungal volatile production has been suggested to play a role in this process (Hulcr & Cognato 2010, Hulcr & Stelinski 2017).

Where mycangia serve the important role of maintaining the close association between beetles and their symbionts, it has been argued that obligatory insect mutualisms involve a one-on-one association between insect and symbiont (Hubbard 1896, Talbot 1977, Cook & Rasplus 2003). Although this might be true for some symbioses, it is not necessarily the case for ambrosia beetles. Until about the 1960s, the ambrosia symbiosis was considered a one-on-one relationship between a beetle species and its

fungal symbiont (Hubbard 1896). This was largely because a single dominant species of fungus could be isolated from both the beetle gallery and mycangium (Gebhardt et al. 2004). However, as culture methods improved and the symbiosis between ambrosia beetles and fungi was better understood, studies revealed that the fungal community within both the gallery and the mycangium was more complicated than initially believed (Batra 1963, Batra 1966, Gebhardt et al. 2004, Na et al. 2018, Carrillo et al. 2019, Six 2019).

Fungi other than the primary symbiont found growing in ambrosia beetle galleries and on which these beetles also feed are referred to as secondary or auxiliary ambrosia fungi (Batra 1966, Batra 1967, Kolařík & Kirkendall 2010). Many of these auxiliary species reside in the same or related genera as the primary ambrosia symbiont, and some species that act as a primary symbiont of one beetle can be an auxiliary symbiont of another species (Batra 1966). It has been suggested that the acquisition of these auxiliary symbionts could occur through cross-contamination of galleries when a single host is infested with multiple species of ambrosia beetle (Gebhardt et al. 2004, Carrillo et al. 2014).

The availability of high-throughput sequencing methods has substantially expanded our understanding of the fungal communities within the mycangia of ambrosia beetles. For example, a recent metagenomic study by Kostovcik et al. (2015) considered the mycangial communities of *Xylosandrus crassiusculus, Xyleborus affinis* and *Xyleborus ferrugineus* and found numerous fungal lineages present in an organ that was originally thought to contain only the primary symbiont. It is clear from this work that our understanding of the community structure associated with and maintained by these beetles remains very limited but also that substantial advances in our understanding of this topic can be expected in the future as powerful new technologies based on genetic data emerge.

7. Fungi associated with ambrosia beetles

Other than a single species of Basidiomycete fungi (Simmons et al. 2016a), all known fungal symbionts of ambrosia beetles are Ascomycetes. The best-known ambrosial fungi are species in the Microascales (Mayers et al. 2018, 2020) and Ophiostomatales (Massoumi-Alamouti et al. 2009, Vanderpool et al. 2018). However, there are also various species of less well-known ambrosial associates that reside in two genera of Hypocreales, *Geosmithia* and *Fusarium* (Kolařík & Kirkendall 2010, Kasson et al. 2013).

Each of these fungal orders accommodates species that have diverse lifestyles, many of which are also important plant pathogens of quarantine concern (Spatafora & Blackwell 1994, Massoumi-Alamouti et al. 2009, Machingambi et al. 2014).

Primary ambrosia fungi are both morphologically and physiologically adapted to their symbiotic lifestyles (Cassar & Blackwell 1996, Klepzig & Six 2004, Kolařík & Kirkendall 2010, Jordal & Cognato 2012). Some of these adaptations include: (a) Spores suited to arthropod dispersal (Cassar & Blackwell 1996); (b) an ability to convert to a yeast-like growth phase known as "ambrosial growth" allowing dispersal via the beetle mycangia (Batra & Michie 1963, Batra 1967); and (c) the production of sweet aromas that attract their beetle vectors (Hulcr et al. 2011). Although numerous species of ambrosia fungi have been described, it is estimated that only 5% have so far been identified (Kolařík & Hulcr 2009, Hulcr & Stelinski 2017).

7.1. Ophiostomatoid associates

The two best-known genera of ambrosia fungi are *Raffaelea* and *Ambrosiella* (Batra 1967, Cassar & Blackwell 1996, Harrington et al. 2010). These genera, along with various others, reside in a group of fungi that have collectively been referred to as the Ophiostomatoid fungi – a term originally established to accommodate the taxonomically confused genera *Ceratocystis* and *Ophiostoma* (Wingfield et al. 1993, Gebhardt et al. 2004). These genera share several convergent morphological traits allowing them to being vectored by various arthropods. This morphological convergence has led to such strong resemblances between species residing in these genera that they could only be clearly resolved when DNA sequencing and phylogenetic inference became available (Cassar & Blackwell 1996). Consequently, some of the first phylogenetic analyses including species in these genera, showed not only that they were not closely related, but that they reside in completely different orders, the Ophiostomatales and Microascales (Hausner et al. 1993, Spatafora & Blackwell 1994, Cassar & Blackwell 1996).

The Ophiostomatales comprises approximately 300 species residing in 12 genera (**Figure 3**) (De Beer et al. 2013b) including the soon to be resurrected *Dryadomyces* (Wilhelm de Beer & Miranda Procter pers. comm.) These species can be found in a wide variety of niches, but the majority are found in

woody substrates. Most species are associated with a variety of bark beetles but around 34 species residing in four genera, *Raffaelea* (Gebhardt & Oberwinkler 2005), *Dryadomyces* (Gebhardt et al. 2005), *Aureovirgo* (Van Der Linde et al. 2016), and *Afroraffaelea* (Bateman et al. 2017), are considered primary ambrosia beetle associates (**Table 1**). Although generally considered more as auxiliary symbionts, two species of *Sporothrix*, five species of *Leptographium* and at least ten species of *Ophiostoma* have also been isolated and described as associates of ambrosia beetles **(Table 1**).

The most common ambrosia beetle symbionts in the Microascales reside in the Ceratocystidaceae (De Beer et al. 2014, Mayers et al. 2015, 2018, 2020) (**Figure 4**). Currently there are 23 described species residing in five genera that are considered primary ambrosia symbionts. They include species of *Ambrosiella* (Brader 1964), *Meredithiella* (Mayers et al. 2015, 2018), *Phialophoropsis* (Batra 1967, Mayers et al. 2015), *Toshionella* and *Wolfgangiella* (Mayers et al. 2020) (**Table 1**). In addition, species of *Endoconidiophora* as defined by De Beer et al. (2014) are primary associates of bark beetles such as species of *Ips* and *Dendroctonus* (Redfern et al. 1987, Krokene & Solheim 1997, Wingfield et al. 1997, Harrington & Wingfield 1998, Marin et al. 2005). However, aside from the Ceratocystidaceae, there are also species in the Gondwanamycetaceae and Graphiaceae (**Table 1)** (Kolařík & Hulcr 2009, Kolařík et al. 2015, Lynch et al. 2016, Na et al. 2018) that are associated with ambrosia beetles and other arthropods, but little is known regarding these species or their relationships with their insect vectors.

7.2. Hypocrealean associates

Two genera in the Hypocreales, *Fusarium* and *Geosmithia*, are known to include primary ambrosia beetle symbionts (Kolařík & Kirkendall 2010, Kasson et al. 2013). Species in these genera are so strongly adapted to their symbiotic lifestyles that they no longer resemble their free-living relatives.

Within the recently revised *Fusarium solani* complex (Geiser et al. 2020), there is a single clade known as the Ambrosia *Fusarium* Clade (AFC) that accommodates 24 species lineages associated with ambrosia beetles in the genus *Euwallacea* (Kasson et al. 2013, Na et al. 2018, Carillo et al. 2019, Lynn et al. 2020, 2021). Unlike other *Fusarium* species that have characteristic fusiform and septate macroconidia, species in the AFC produce clavate macroconidia, apparently an adaptation to their

symbiotic relationships (Freeman et al. 2013, Kasson et al. 2013). The unique morphology of the macroconidia in the *Fusarium* spp. associated with *Xyleborus fornicatus* (syn = *Euwallaceae fornicatus*) resulted in its initial description in the genus *Monacrosporium* (Gadd & Loos, 1947). Some forty years later, this species was reidentified to be a *Fusarium* and was described as the first AFC member and given the name *Fusarium ambrosium* (Brayford 1987, Nirenberg 1990). It would not be until 20 years later that Kasson et al. (2013) showed that several AFC species exist, all associated with different species of *Euwallacea* ambrosia beetles. Of the 24 recognized lineages in the AFC clade, only 13 have been formally described as species (**Table 1**) (Nirenberg 1990, Freeman et al. 2013, Kasson et al. 2013, Aoki et al. 2018, 2019, Na et al. 2018, Lynn et al. 2020, 2021). The remaining 11 lineages are identified by their respective AF lineage numbers introduced sequentially at the time when they were first recognized (**Figure 5**).

Species of *Geosmithia* are best known as associates of phloem feeding bark beetles (Kolařík & Kirkendall 2010, Hulcr & Stelinski 2017). Very few species display different lifestyles, however four species have been described as symbionts of South American ambrosia beetles (Kolařík & Kirkendall 2010, Machingambi et al. 2014, Kolařík et al. 2015)(**Table 1**). Currently, ambrosia beetle diversity and that of their fungi are poorly known in the Southern Hemisphere, and *Geosmithia* spp. have only been identified as primary ambrosia beetle symbionts in the neotropics. Because of their prevalence in the galleries of these beetles, it has been hypothesized that they may serve as the dominant symbionts in this region (Hulcr & Stelinski 2017).

7.3. Basidiomycete associates

To date, only a single basidiomycete species has been described as a primary associate of ambrosia beetles. *Flavodon ambrosius* is a white-rot polypore described in 2016 as the primary symbiont of an exotic ambrosia beetle, *Ambrosiodmus* (*Amb.*) *lecontei,* introduced into the USA from Asia (Li et al. 2015, Simmons et al. 2016). Later that same year, *F. ambrosius* was also found to be the associated with *Ambrosiophilus* (*Amp.*) *atratus,* a species closely related to *Ambrosiodmus,* also introduced into the USA from Asia (Kasson et al. 2016). However, *F. ambrosius* is not restricted to the USA and has now been found to be the primary symbiont of at least five ambrosia beetle species - *Amb. lecontei,*

Amb. minor, Amb. rubricollis, Amp. atratus and *Amp. subneptotulus* - from four different countries i.e. USA, China, South Korea, and Vietnam (Simmons et al. 2016, Kasson et al. 2016, Li et al. 2017).

Unlike most Ascomycete symbionts of ambrosia beetles, *F. ambrosius* is capable of lignocellulose degradation (De Fine Licht & Biedermann 2012, Kasson et al. 2016). This ability provides its associated ambrosia beetle species with a significant ecological advantage. Where Ascomycete associated beetles need to infest freshly decaying wood to enable their fungi to acquire the nutrients they need, *F. ambrosius* can persist long after the death of a tree. This persistence in turn allows multiple beetle generations to brood in the same host, whereas most Ascomycete associated ambrosia beetles can only complete a single generation in a host before needing to disperse (Li et al. 2015, Kasson et al. 2016). In relatively few studies, many contrasts have already been revealed between the Ascomycete and Basidiomycete ambrosia symbioses, which challenges what can be considered the "classical paradigm" of the ambrosia symbiosis.

7.4. Yeast associates

It has been well documented that a wide variety of yeasts associate with ambrosia beetles (Batra 1966, Van Der Walt 1972, Kinuura 1995). The composition of yeast species found within the gallery systems is highly variable, but some commonly isolated genera are *Ambrosiozyma, Candida, Ogataea,* and *Pichia* (Van Der Walt 1972, Kinuura 1995, Endoh et al. 2011, Yun et al. 2015, Li et al. 2018, Saucedo-Carabez et al. 2018). However, unlike many of the other ambrosia fungi, most yeasts are often considered non-specific auxiliary ambrosia symbionts (Nakashima et al. 1986, Kinuura 1995, Hulcr & Stelinski 2017). Although this may be true for many species, it does appear that at least some have a more intimate relationship with beetle partners (Saucedo-Carabez et al. 2018).

Despite extensive study, we still know very little about the role of yeasts in the ambrosia symbiosis (Li et al. 2018, Saucedo-Carabez et al. 2018, Ibarra-Juarez et al. 2020). What has been noted in a few studies was that their composition varies during brood succession (Batra 1966, Nakashima et al. 1986, Kajimura & Hijii 1992, Ibarra-Juarez et al. 2020). These yeast species are likely the initial colonizers of the ambrosial gallery being succeeded by the primary symbiont (Cruz et al. 2019, Ibarra-Juarez et al. 2020), likely around the time the beetle larvae reach the peak of the developmental stage and require

the most nutrients (Kajimura & Hijii 1992, Kinuura et al. 1995, Cruz et al. 2018). As the brood ages, more variation can be found in the composition of fungi growing in the galleries and it appears as though the adults feed more indiscriminately, supplementing their diets with a variety of auxiliary fungi, including these yeasts (Batra 1966, Nakashima et al. 1986, Freeman et al. 2015).

8. Ambrosia beetles and their fungi may not be agents of wood decay

Early literature on bark and ambrosia beetles led to a common view that these insects and their fungal symbionts are involved in the process of wood decay. However, even early research suggested that this might not actually be true (Gibbs 1993). In fact, in some instances the presence of ambrosia beetles and their symbionts within wood may extend its durability by outcompeting saprophytic, wood-degrading microorganisms (Skelton et al. 2019b, 2019c).

Ambrosia beetles are not known to consume wood. Instead, they rely on their symbionts to concentrate the nutrients from within the woody tissues of their host into a usable form that they can consume. However, numerous studies have also shown that the Ophiostomatoid fungi associated with these insects are not able to degrade the complex molecules which make up the main components of wood (Gibbs 1993, Valiev et al. 2009, De Fine Licht & Biedermann 2012, Six 2019). Therefore, the question then remains as to how ambrosia fungi acquire their nutrients.

To resolve the question as to how these fungi acquire nutrients from the host, one of the most important factors that must be considered is how ambrosia fungi colonize the host. Multiple studies using artificial inoculation of Ophiostomatoid fungi on various hosts have revealed how this happens. Overall, these studies show that when the ambrosia beetles begin to construct their galleries, during the early stages of tree death, the wood contains large amounts of easily accessible substrates such as free sugars (De Fine Licht & Biedermann 2012, Kasson et al. 2016, Skelton et al. 2019b). Therefore, to obtain these nutrients using a route of least resistance, the inoculated Ophiostomatoid fungi enter the host through mechanical damage, induced either by the beetles during gallery construction or during artificial inoculation processes (Ballard et al. 1984, Takahashi et al. 2010). Colonization of the host by the fungi continues either with the fungus moving from tracheid to tracheid, through medullary rays via bordered pits, or occasionally by mechanical force, with little to no indication of enzymatic activity facilitating

the process (Ballard et al. 1984, Gibbs 1993, Six 2019). Because little or no enzymatic activity is involved to break down the lignified tissue of the wood, fully intact remnants of the cells collapse when drained of their contents (Ballard et al. 1984). The large availability of accessible free nutrients found in these cells then enable the fungi to proliferate extensively within them (Ballard et al. 1984, Kajii et al. 2013).

While most Ascomycete ambrosia fungi enter and colonize the host through wounds induced by their beetle associates and can therefore not be considered primary agents of wood decay, there are known exceptions. Two white-rot Basidiomycete species, *Flavodon ambrosius* (Polyporales) (Simmons et al. 2016) and *Phlebiopsis gigantea* (Six 2019), respectively isolated from *Ambrosiodmus* and *Ambrosiophilus* ambrosia beetles and the bark beetle *Dendroctonus brevicormis*, are regarded as primary nutritional symbionts. Unlike the Ascomycetes, both these fungi have been found to produce the enzymes necessary for lignin degrading (Harrington 2005, Kasson et al. 2016). However, due to the small number of known associations between bark and ambrosia beetles and Basidiomycete fungi, these have so far been minimally studied.

9. Global movement of ambrosia beetles

The increased speed and volume of international trade has resulted in the movement of exotic pest species becoming very real threat to global biodiversity, food security and forestry (Colunga-Garcia et al. 2009, Kirkendall & Faccoli 2010, Wingfield et al. 2015, Fahrner & Aukema 2018). Curculionidae beetles, especially bark and ambrosia beetles, are some of the most frequently intercepted exotic pests at global ports of entry (Haack 2001,2006, Brockerhoff et al. 2006, Colunga-Garcia et al. 2009, Kirkendall & Faccoli 2010). The ease of movement of these beetles is commonly attributed to their small size and their association with various wood products including plants moved around for the living plant trade and improperly treated wood shipping materials (Haack 2001,2006, Brockerhoff et al. 2006, Kirkendall & Faccoli 2010, Marini et al. 2011, Muerisse et al. 2019).

In their native range, ambrosia beetles are not considered to be of great economic importance and are generally treated as secondary pests. This is consistent with the fact that in more than 95% of ambrosia beetles together with their fungal symbionts are harmless, infesting only stressed, dying or recently dead

trees (Hulcr & Stelinski 2017). In many cases, even when introduced into a new range, ambrosia beetles remain harmless. However, there are numerous and growing examples where these insects have switched to infest healthy trees. This conversion to primary pest status arises from an 'evolutionary mismatch', where the beetles become both ecologically and economically damaging (Hulcr & Dunn 2011, Hulcr et al. 2017, Hulcr & Stelinski 2017, Na et al. 2018).

Because ambrosia beetles are relatively inconspicuous and usually found on stressed or dying trees, invasions have attracted limited research attention, at least as compared to their bark beetle relatives. In their extensive review, Ploetz et al. (2013) highlighted some of the most devastating invasions recorded for both bark and ambrosia beetles. Yet these authors were able to highlight only four serious ambrosia beetle invasions. These included *Xyleborus glabratus* and its symbiont *Raffaelea lauricola* causing laurel wilt on avocado and other Lauraceae species in the USA (Harrington et al. 2008); *Platypus quercivora* and its symbiont *R. quercivora* affecting species of Fagaceae in Japan (Kubono & Ito 2002); *Platypus koryoensis* and its symbiont *R. quercus-mongolicae* infesting *Quercus* species in Korea (Kim et al. 2009); and the *Euwallacea fornicatus* species complex and their *Fusarium* symbionts affecting a wide range of tree species in both the Western and Eastern Hemispheres (Freeman et al. 2013, Kasson et al. 2013).

A long-standing goal of invasion biologists is to highlight the importance of native range studies in understanding and predicting the nature of invasion (Liebhold et al. 1995, Kolar & Lodge 2001, Sakai et al. 2001, Broennimann & Guisan 2008, Hulcr et al. 2017). This is relevant because many insect species only become noteworthy after they have invaded into a non-native range and become problematic. This is unfortunate because a substantial understanding of their potential as invasive pests can be revealed from studying them in their native ranges, as has been found with beetles of the *E. fornicatus* species complex (Hulcr et al. 2017). As globalization is not going to slow down any time soon, researchers will need to shift their attention to native range studies to ensure sustainable forestry and agriculture in the long run.

10. The Polyphagous Shot Hole Borer

Since the publication of Ploetz *et al* (2013), there have been few records of new and damaging ambrosia beetle invasions. However, the devastation caused by *E. fornicatus*, colloquially known as the Polyphagous Shot Hole Borer, has continued to spread to new areas of the world. For example, in 2017 both *E. fornicatus* and its closely related sister species *E. kuroshio* (commonly known as the Kuroshio Shot Hole Borer) had become established in the USA (Stouthamer et al. 2017, Na et al. 2018). The following year, *E. fornicatus* was reported for the first time from South Africa (Paap et al. 2018) where it is causing very substantial damage on both indigenous and exotic tree species (Potgieter et al. 2020, FABI 2021).

Euwallacea fornicatus is a Xyleborine ambrosia beetle native to Asia (Stouthamer et al. 2017). Encountered outside of its native range for the first time in 2003, the species was disregarded because the damage observed was minimal or its infestations occurred on species of little importance (Rabgalia et al. 2006, Mendel et al. 2012, Umeda et al. 2016). However, these assumptions soon turned out to be incorrect when it became clear that the species is highly polyphagous, and has now been found to infest more than 200 different ecologically and economically important tree species (Eksalen et al. 2013, FABI 2021).

Despite being attracted to such a large variety of hosts, it is not the beetle itself that causes most of the devastation attributed to it. Like most other ambrosia beetles, *E. fornicatus* associates with a primary nutritional symbiont, which in its case is a member of the AFC, *Fusarium euwallaceae* (Freeman et al. 2012, 2013). Unlike many other ambrosia symbionts, *F. euwallaceae* is an aggressive tree pathogen, able to colonize and kill a host even in the absence of its beetle partner, as is often seen in non-reproductive hosts (Eksalen et al. 2013). In cases where the fungal symbiont is highly pathogenic, the effects of *E. fornicatus* invasion, although delayed, can be severe and consequently result in large economic losses such as those observed in the USA and Israel avocado industries (Mendel et al. 2012, Eksalen et al. 2012, 2013).

11. Ambrosia beetles in South Africa

The large-scale losses to global forests, timber and fruit tree industries caused by invasive bark and ambrosia beetles has meant that these insects are drawing increasing research interest in many parts of the world (Ploetz et al. 2013). Many serious tree disease outbreaks such as Dutch elm disease, Laurel wilt, thousand cankers disease and Oak wilt can be attributed to the movement of Scolytine bark and ambrosia beetles (Fraedrich et al. 2008, Harrington et al. 2008, Juzwik et al. 2008, Kolarik et al. 2011, Carrillo et al. 2012, Kristis 2013, Ploetz et al. 2013). As bark and ambrosia beetles are still some of the most frequently intercepted pests at ports of entry globally, for example representing 60% of the wood associated insect interceptions in the USA between 1985 and 2000 (Haack 2006), the threat they pose remains great. Although most interceptions recorded are for species of bark beetle, a variety of Xyleborine ambrosia beetles are also frequently encountered (Haack 2001, 2006, Brockerhoff et al. 2006).

In many Southern Hemisphere countries, such as South Africa, these insects remain largely unstudied. This is highlighted by the fact that only three new species of ambrosia beetle, *Cyrtogenius africus* (Van Der Linde et al. 2016), *E. fornicatus* (Paap et al. 2018), and *Xylosandrus crassiusculus* (Nel et al. 2020) have been formally reported from the South Africa subsequent to the passing of Karl Eduard Schedl (1898-1979), who, during his nearly 50 year career, published numerous checklists and described at least 15 new bark and ambrosia beetle species from the country (Schedl 1955, 1960, 1965, 1970, 1975, 1982). The same is also true for the fungal symbionts of these insects, with only six beetles having symbionts known in the country. These include *Ambrosiella roeperi*, *Aureovirgo volantis*, *Fusarium euwallaceae*, *Ophiostoma thermarum*, *Raffaelea albimanens*, *R. hennebertii*, *R. arxii* and *Sporothrix aemulophila* (**Table 2**).

The recent appearance of the aggressive Polyphagous Shot Hole Borer (**Figure 6**) in South Africa has highlighted the vulnerability of forests and tree-based industries to damage by ambrosia beetles (Paap et al. 2018, Smith et al. 2019). The relatively poorly regulated and problematic quarantine policies regarding this category of insects has also become very evident (Paap et al. 2020). This is largely due to (i) the limited phytosanitary measures in place at ports of entry; (ii) the lack of trained personnel to

assist in the detection of these insects and; (iii) a lack in available resources and appropriate funding to improve either these problems (Parliament of the Republic of Southern Africa 2017). Yet, a good starting point to improve regulation and to provide informed and effective quarantine policy for ambrosia beetles, is to create updated records of the species present in the country. The last complete record of bark and ambrosia beetle species present in South Africa was listed in the global catalogue of bark and ambrosia beetle species produced by Wood and Bright (1992). Working through this catalogue, 163 species can be found recorded from South Africa. Of these 20 species reside in the Platypodinae and 143 reside in the Scolytinae (Wood & Bright 1992). However, as this publication is nearly 30 years old, it highlights an urgent need to address current knowledge gaps on the biodiversity of these insects.

The physical devastation being caused by the Polyphagous Shot Hole Borer in South Africa is of great concern with the epidemic continuing to develop and spread. Another important aspect of this invasion is how it has led to a change in the way most South Africans consider forests and forest research. Damage caused by the beetle is most evident in urban landscapes rather than in commercial forests, and this has clearly shifted the view and perception of the local public regarding forestry research to their own properties, gardens, streets, and communities. This is largely because most of the 100 tree species identified as hosts of the beetle in South Africa are ornamental trees. Presently, only commercially planted pecan nut trees (*Carya illinoinensis*) have been reported to be severely affected by this insect (Fell & De Beer, 2019). There are records of other tree species of agricultural and forestry importance, such as avocado and wattle, that are susceptible to infestation by the Polyphagous Shot Hole Borer (Van Den Berg et al. 2019, FABI 2021), but thus far these reports are only represented by trees planted in private gardens or by the roadside. A current view is that South Africa will need to find a means to control the Polyphagous Shot Hole Borer if streets lined with ancient London plane (*Platanus x acerifolia*) and English oak (*Quercus robur*), common sights in areas such as Johannesburg and Knysna, are to be preserved in their current beauty.

12. Conclusions

Symbioses with microbes are widespread among the insects, but insect fungiculture is the most sophisticated mutualism known in nature. The symbiosis between ambrosia beetles and their fungal associates is the oldest and most diverse form of insect fungiculture known. Yet it is also the least well understood of the ant, termite, and beetle farmers. With increasing concern of the global movement of these insects and the threat they pose to sustainable forestry and agricultural practices, these tiny wood borers are drawing increasing commercial and research interest. However, a general lack of knowledge of ambrosia beetles in their native ranges, and largely inadequate policy and regulations to prevent the movement of these insects in many parts of the world implies that they are likely to become increasingly important in the future. There are many aspects in the ambrosia symbiosis that is still poorly understood and additional research into their biodiversity, biology, ecology, and evolution is needed for effective understanding and control of these insects.

Table 1. Sordariomycete fungi with ambrosia beetle associations mentioned in their species descriptions

Order	Species name	Locality	Symbiont	Common name of symbiont	Reference
Hypocreales	*Fusarium ambrosium* [AF-1]	India & Sri Lanka	*Euwallacea fornicatus*	Polyphagous Shot Hole Borer	Nirenberg, 1990
Hypocreales	*Fusarium akasia* [AF-20]	Indonesia	*Euwallacea perbrevis* & *Euwallacea similis*	Tea Shot Hole Borer/-	Lynn et al. 2021
Hypocreales	*Fusarium awan* [AF-21]	Indonesia	*Euwallacea similis*	-	Lynn et al. 2021
Hypocreales	*Fusarium euwallaceae* [AF-2]	Israel & USA	*Euwallacea fornicatus*	Polyphagous Shot Hole Borer	Freeman et al. 2013
Hypocreales	*Fusarium floridanum* [AF-3]	USA	*Euwallacea interjectus*	-	Aoki et al. 2019
Hypocreales	*Fusarium oligoseptatum* [AF-4]	USA	*Euwallacea validus*	-	Aoki et al. 2018
Hypocreales	*Fusarium kuroshium* [AF-12]	Taiwan & USA	*Euwallacea kuroshium*	Kuroshio Shot Hole Borer	Na et al. 2018
Hypocreales	*Fusarium mekan* [AF-22]	Indonesia	*Euwallacea similis*	-	Lynn et al. 2021
Hypocreales	*Fusarium obliquiseptatum* [AF-7]	Australia	*Euwallacea* sp.	-	Aoki et al. 2019
Hypocreales	*Fusarium rekanum* [AF-19]	Indonesia	*Euwallacea perbrevis*	Tea Shot Hole Borer	Lynn et al. 2020
Hypocreales	*Fusarium tuarense* [AF-5]	Malaysia	Unknown	-	Aoki et al. 2019
Hypocreales	*Fusarium variasi* [AF23]	Indonesia	Unknown	-	Lynn et al. 2021
Hypocreales	*Fusarium warna* [AF-24]	Indonesia	*Euwallacea perbrevis*	Tea Shot Hole Borer	Lynn et al. 2021
Hypocreales	*Geosmithia cnesini*	Costa Rica	*Cnesinus lecontei*	-	Kolarik et al. 2015
Hypocreales	*Geosmithia eupagioceri*	Costa Rica	*Eupagiocerus dentipes*	-	Kolarik & Kirkendall 2010
Hypocreales	*Geosmithia rufescens*	Costa Rica	*Cnesinus lecontei* *Eupagiocerus dentipes*	- -	Kolarik & Kirkendall 2010
Hypocreales	*Geosmithia microcorthyli*	Costa Rica	*Microcorthylus* sp.	-	Kolarik & Kirkendall 2010
Microascales	*Ambrosiella batrae*	USA	*Anisandrus sayi*	-	Mayers et al. 2015
Microascales	*Ambrosiella beaveri*	USA	*Cnestus mutilatus*	Camphor Shot Borer	Six et al. 2009
Microascales	*Ambrosiella catenulata*	Taiwan	*Anisandrus apicalis*	-	Lin et al. 2017
Microascales	*Ambrosiella cleistominuta*	USA	*Anisandrus maiche*	-	Mayers et al. 2017
Microascales	*Ambrosiella grosmanniae*	USA	*Xylosandrus germanus*	Black Stem Borer	Mayers et al. 2015
Microascales	*Ambrosiella hartigii*	Germany	*Anisandrus dispar*	European Shot Hole Borer	Batra 1967
Microascales	*Ambrosiella nakashimae*	USA	*Xylosandrus amputatus*	-	Mayers et al. 2015

Microascales	*Ambrosiella remansi*	Madagascar	*Remansus mutabilis*	-	Mayers et al 2020
Microascales	*Ambrosiella roeperi*	USA	*Xylosandrus crassiusculus*	Granulate Ambrosia Beetle	Harrington et al. 2014
Microascales	*Ambrosiella xylebori*	Ivory Coast	*Xyleborus compactus*	Black Twig Borer	Brader 1964
Microascales	*Knoxdaviesia scolytodes*	Costa Rica	*Scolytodes unipuncatatus*	-	Kolarik & Hulcr, 2009
Microascales	*Meredithiella fracta*	USA	*Corthylus populans*	Chestnut Timber Worm	Mayers et al 2018
Microascales	*Meredithiella guianensis*	French Guiana	*Corthylus* sp.	-	Mayers et al 2018
Microascales	*Meredithiella norrisii*	USA	*Corthylus punctatissimus*	Pitted Ambrosia Beetle	Mayers et al. 2015
Microascales	*Phialophoropsis ferruginea*	Germany	*Trypodendron lineatum*	Striped Ambrosia Beetle	Mayers et al. 2015
Microascales	*Phialophoropsis hubbardii*	USA	*Trypodendron retusum*	-	Mayers et al. 2020b
Microascales	*Phialophoropsis leachii*	USA	*Trypodendron betulae*	-	Mayers et al. 2020b
Microascales	*Phialophoropsis nunbergii*	Germany	*Trypodendron domesticum*	European Hardwood Ambrosia Beetle	Mayers et al. 2020b
			Trypodendron signatum	-	
Microascales	*Phialophoropsis trypodenri*	USA	*Trypodendron scabricollis*	-	Mayers et al. 2015
Microascales	*Toshionella nipponensis*	Japan	*Scolytoplatypus shogun*	-	Mayers et al 2020
Microascales	*Toshionella taiwanensis*	Taiwan	*Scolytoplatypus* sp.	-	Mayers et al 2020
Microascales	*Toshionella transmara*	Japan	*Scolytoplatypus* sp.	-	Mayers et al 2020
			Xylosandrus crassiusculus	Granulate Ambrosia Beetle	
Microascales	*Wolfgangiella franznegeri*	South Africa	*Scolytoplatypus fasciatus*	-	Mayers et al 2020
Microascales	*Wolfgangiella madagascariensis*	South Africa	*Scolytoplatypus permirus*	-	Mayers et al 2020
Microascales	*Graphium euwallaceae*	USA	*Euwallaceae fornicatus*	Polyphagous Shot Hole Borer	Lynch et al. 2016
Microascales	*Graphium kuroshium*	USA	*Euwallacea kuroshium*	Kuroshio Shot Hole Borer	Na et al. 2018
Microascales	*Graphium scolytodes*	Costa Rica	*Scolytodes unipunctatus*	-	Kolarik et al. 2015
Ophiostomatales	*Afroraffaelea ambrosiae*	USA	*Premnobius cavipennis*	-	Bateman et al. 2017
Ophiostomatales	*Aureovirgo volantis*	South Africa	*Cyrtogenius africus*	-	Van Der Linde et al. 2016
			Amasa concitatus	-	
Ophiostomatales	*Drydomyces amasae*	Taiwan	*Amasa aff. glaber*	-	Gebhardt et al. 2005
Ophiostomatales	*Leptographium flavum*	Poland	*Anisandrus dispar*	European Shot Hole Borer	Jankowaik et al. 2018
Ophiostomatales	*Leptographium gestamen*	Argentina	*Gnathotrupes* sp.	-	De Errasti et al. 2016
Ophiostomatales	*Leptographium tardum*	Norway, Poland	*Trypodendron domesticum*	European Hardwood Ambrosia Beetle	Jankowaik et al. 2018

			Trypodendron signatum	-	
Ophiostomatales	*Leptographium trypodendroni*	Poland	*Trypodendron domesticum*	European Hardwood Ambrosia Beetle	Jankowaik et al. 2017
Ophiostomatales	*Leptographium verrucosum*	Germany	*Xyleborus dryographus*	-	Gebhardt et al. 2002
Ophiostomatales	*Ophiostoma brevicolle*	USA	*Trypodendron retusus*	-	Davidson 1958
Ophiostomatales	*Ophiostoma canum*	Finland	*Trypodendron lineatum*	Striped Ambrosia Beetle	Linnakoski et al. 2010
Ophiostomatales	*Ophiostoma carpernteri*	USA	*Trypodendron lineatum*	Striped Ambrosia Beetle	Hausner et al. 2003
Ophiostomatales	*Ophiostoma denticulata*	USA	*Gnathotrichus denticulatus*	-	Davidson 1979
Ophiostomatales	*Ophiostoma distortum*	USA	unknown ambrosia beetle	-	Davidson 1971
Ophiostomatales	*Ophiostoma longicollum*	Japan	*Platypus quercivorus*	Oak Ambrosia Beetle	Masuya et al. 1998
Ophiostomatales	*Ophiostoma patagonicum*	Argentina	unknown ambrosia beetle	-	De Errasti et al. 2016
Ophiostomatales	*Ophiostoma rachisporum*	Finland	*Trypodendron lineatum*	Striped Ambrosia Beetle	Linnakoski et al. 2010
Ophiostomatales	*Ophiostoma solheimii*	Poland	*Anisandrus dispar*	European Shot Hole Borer	Jankowaik et al. 2019
Ophiostomatales	*Ophiostoma thermarum*	South Africa	*Cyrtogenius africus*	-	Van Der Linde et al. 2016
Ophiostomatales	*Ophiostoma torulosum*	Germany	*Trypodendron domesticum*	European Hardwood Ambrosia Beetle	Butin & Zimmerman 1972
Ophiostomatales	*Raffaelea aguacate*	USA	*Xyleborinus bispinatus*	-	Saucedo-Carabez et al. 2018
Ophiostomatales	*Raffaelea albimanens*	South Africa	*Crossotarsus externedentatus*	Exotic Pin-Hole Borer	Scott & du Toit 1970
Ophiostomatales	*Raffaelea arxii*	South Africa	*Xyleborus torquatus*		Scott & du Toit 1970
			Xyleborus glabratus	Redbay Ambrosia Beetle	
Ophiostomatales	*Raffaelea brunnea*	Unknown	*Monarthrum* sp.	-	Harrington et al. 2010
Ophiostomatales	*Raffaelea campbellii*	USA	*Xyleborus glabratus*	Redbay Ambrosia Beetle	Simmons et al. 2016b
Ophiostomatales	*Raffaelea canadensis*	Canada	*Gnathotrichus sulcatus*	Western Hemlock Wood Stainer	Harrington et al. 2010
			Platypus wilsoni	Wilson's White-Headed Ambrosia Beetle	
			Crossotarsus externedentatus	Exotic Pin-Hole Borer	
Ophiostomatales	*Raffaelea crossotarsa*	Taiwan	*Crossotarsus emancipatus*	-	Simmons et al. 2016
Ophiostomatales	*Raffaelea cyclorhipidia*	Taiwan	*Cyclorhipidion ohnoi*	-	Simmons et al. 2016
Ophiostomatales	*Raffaelea ellipticospora*	USA	*Xyleborus glabratus*	Redbay Ambrosia Beetle	Harrington et al. 2010
Ophiostomatales	*Raffaelea fusca*	USA	*Xyleborus glabratus*	Redbay Ambrosia Beetle	Harrington et al. 2010
Ophiostomatales	*Raffaelea gnathotrichi*	USA	*Gnathotrichus retusus*	Western Pinewood Stainer	Harrington et al. 2010
Ophiostomatales	*Raffaelea hennebertii*	South Africa	*Crossotarsus externedentatus*	Exotic Pin-Hole Borer	Scott & du Toit 1970

Ophiostomatales	*Raffaelea lauricola*	USA	*Xyleborus glabratus*	Redbay Ambrosia Beetle	Harrington et al. 2008
Ophiostomatales	*Raffaelea montetyi*	Europe	*Platypus cylindrus*	Oak Pinhole Borer	Gebhardt et al. 2004
			Xyleborus monographus	Mediterranean Oak Borer	
			Xyleborus dryographus	-	
Ophiostomatales	*Raffaelea quercivora*	Japan	*Platypus quercivorus*	Oak Ambrosia Beetle	Kubono et al. 2002
Ophiostomatales	*Raffaelea quercus-mongolicae*	South Korea	*Platypus koryoensis*	-	Kim et al. 2009
Ophiostomatales	*Raffaelea rapanea*	South Africa	Platypodinae sp.	-	Musvuugwa et al. 2015
Ophiostomatales	*Raffaelea santoroi*	Argentina	*Platypus sulcatus*	-	Guerrero 1966
			Euplatypus camaldulensis	-	
Ophiostomatales	*Raffaelea scolytodis*	Costa Rica	*Scolytodes unipunctatus*	-	Kolarik & Hulcr2009
Ophiostomatales	*Raffaelea seticollis*	USA	unknown ambrosia beetle	-	Musvuugwa et al. 2015
Ophiostomatales	*Raffaelea subalba*	USA	*Xyleborus glabratus*	Redbay Ambrosia Beetle	Harrington et al. 2010
Ophiostomatales	*Raffaelea subfusca*	USA	*Xyleborus glabratus*	Redbay Ambrosia Beetle	Harrington et al. 2010
Ophiostomatales	*Raffaelea sulcati*	Canada	*Gnathotrichus sulcatus*	Western Hemlock Wood Stainer	Funk 1970
Ophiostomatales	*Raffaelea sulphurea*	USA	*Xyleborinus saxesenii*	Fruit-Tree Pinhole Borer	Harrington et al. 2010
Ophiostomatales	*Raffaelea tritirachium*	USA	*Monarthrum mali*	Apple Wood Stainer	Batra 1967
Ophiostomatales	*Raffaelea vaginata*	South Africa	*Lanurgus* sp.	-	Musvuugwa et al. 2015
Ophiostomatales	*Raffaelea xyleborina*	USA	*Xyleborinus andrewesii*	-	Simmons et al. 2016b
Ophiostomatales	*Raffealea ambrosiae*	UK	*Platypus* spp.	-	Batra 1967
Ophiostomatales	*Raffealea borbonica*	La Reunion	unknown ambrosia beetle	-	Procter et al. 2020
Ophiostomatales	*Sporothrix aemulophila*	South Africa	*Xyleborinus aemulus*	-	Musvuugwa et al. 2015
Ophiostomatales	*Sporothrix cabralii*	Argentina	*Gnathotrupes*	-	De Errasti et al. 2016

Table 2. Ambrosia beetle species with known symbionts reported from South Africa

Beetle species	Locality	Symbiont	Reference
Cyrtogenius africus	Limpopo	*Ophiostoma thermarum*	Van Der Linde et al. 2016
		Aureovirgo volantis	
Euwallaceae fornicatus	Natal	*Fusarium euwallaceae*	Paap et al. 2018
Crossotarsus externedentatus	Natal	*Raffaelea albimanens*	Scott & Du Toit, 1970
		Raffaelea hennebertii	
Xyleborinus aemulus	Western Cape	*Sporothrix aemulophila*	Musvuugwa et al. 2015
Xyleborus torquatus	Natal	*Raffaelea arxii*	Scott & Du Toit, 1970
Xylosandrus crasssiuculus	Limpopo	*Ambrosiella roeperi*	Nel et al. 2020

Figure 1. Some common ambrosia and bark beetles. (A-C) Ambrosia beetles (A) *Xylosandrus crassiusculus*, (B) *Xyleborinus saxesenii*, (C) *Scolytoplatypus fasciatus.* (D-E) Bark beetles (D) *Orthotomicus erosus*, (E) *Hylastes angustatus*, (F) *Hypothenemus eruditus.*

Figure 2. Differences in gallery construction of bark and ambrosia beetles. (A) Fallen log infested with bark and ambrosia beetles. (B) Ambrosia beetle gallery constructed deep into the sapwood of an infested pecan branch. (C) Bark beetle gallery constructed superficially in the cork cambium layer of an infested log.

Figure 3. Maximum likelihood phylogeny of the Ophiostomataceae recreated using LSU data from the revision of the class by De Beer et al. (2013) and including additional genera included in the family by Gebhardt et al. (2005), Van Der Linde et al. (2013) and Bateman et al. (2017). Specific genera associated with ambrosia beetles are indicated in bold and the beetle associated (if known) is given below the genus name. Bootstrap values above 70% are shown at nodes.

Figure 3

Ophiostomataceae
28S
Mega7
1000x Bootstrap
769bp

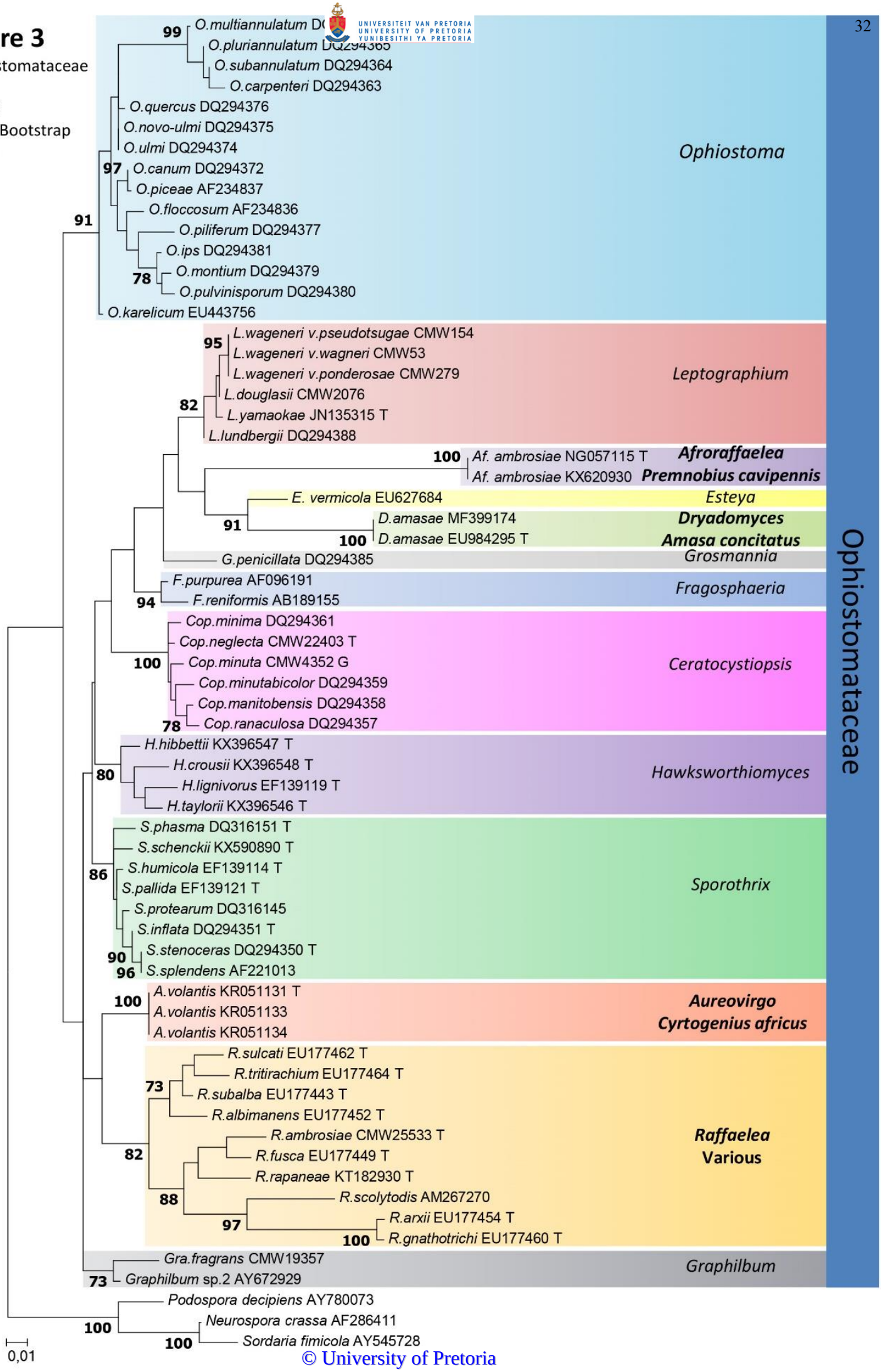

Figure 4. Maximum likelihood phylogeny of the Ceratocystidaceae recreated using multigene data from Nel et al. (2018) and Mayers et al. (2020). Specific genera associated with bark and ambrosia beetles are indicated in bold and the genus name of the associated beetle (if known) is given below the genus name. Bootstrap values above 70% are shown at nodes.

Figure 4

Ceratocytidaceae
28S_60S_ITS_MCM7
Mega7
1000x Bootstrap
2209bp

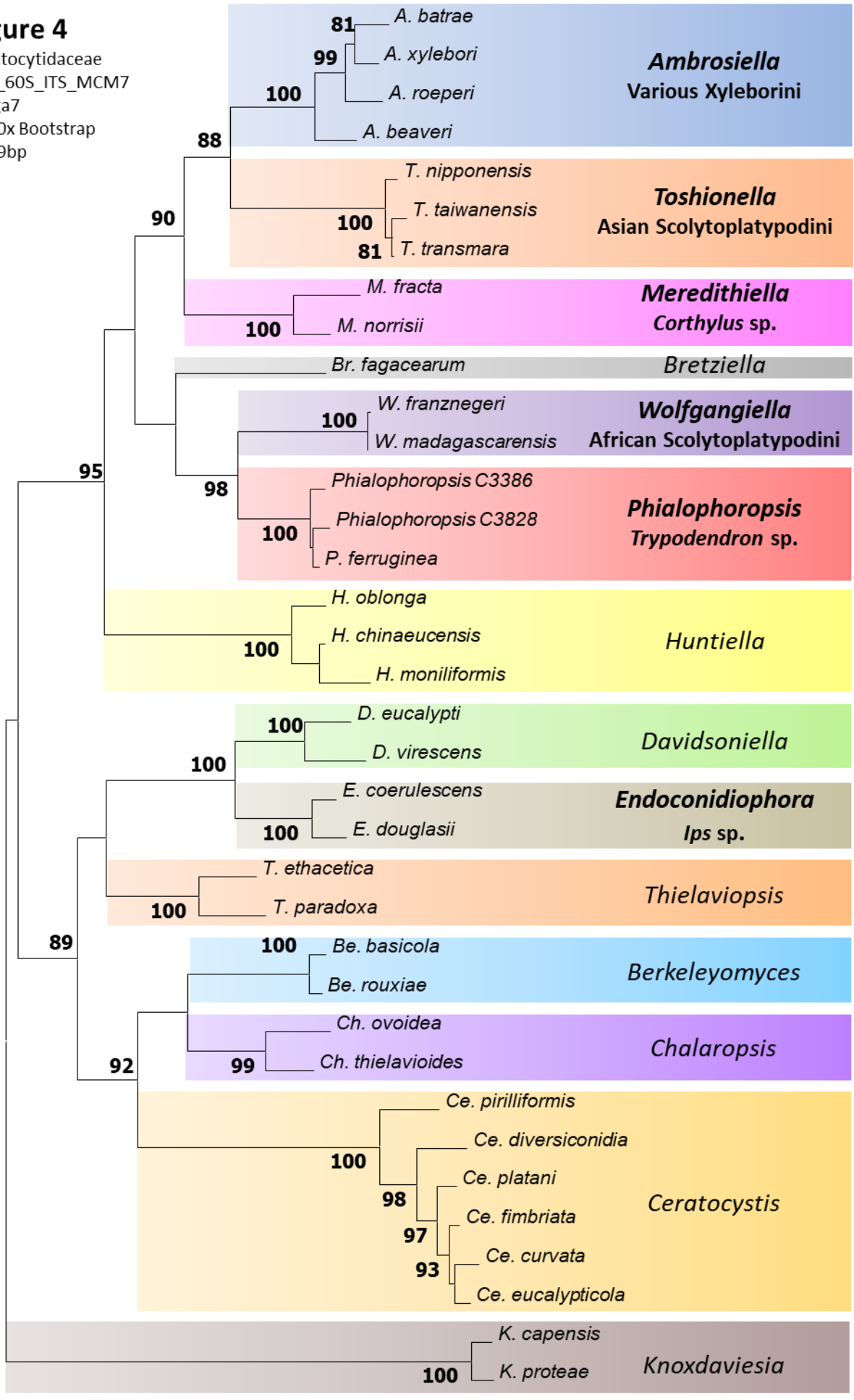

Figure 5. Maximum likelihood phylogeny of the Ambrosia *Fusarium* Clade recreated using multigene data from Lynn et al. (2020). Named species lineages are indicated in bold and the species name of the associated beetle (if known) is given below the fungal species name. AF numbers for both named and unnamed species lineages are given in brackets. Bootstrap values above 70% are shown at nodes. [Note: As of 13 April 2021, the DNA sequence data used by Lynn et al. 2021 had not yet been made publicly available and were therefore not included in the phylogenetic analyses].

Figure 5

Ambrosia *Fusarium* Clade
RPBII_ITS_EF
Mega7
1000x Bootstrap
2305bp

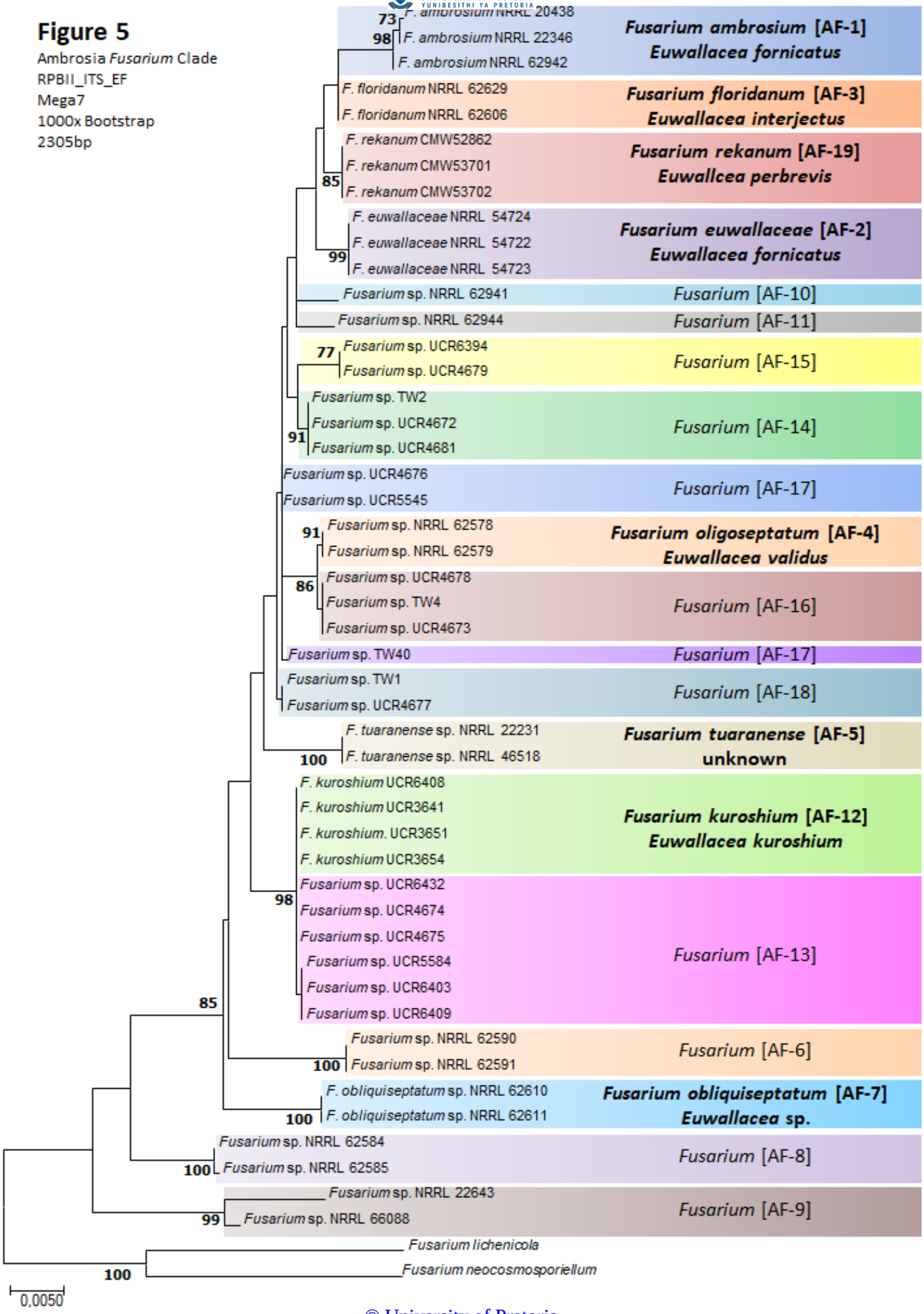

Figure 6. Male (small) and female (large) Polyphagous Shot Hole Borer beetles. Scale = 1 mm.

References

Aanen, D.K., Eggleton, P., Rouland-Lefevre, C., Guldberg-Frøslev, T., Rosendahl, S. & Boomsma, J.J. (2002). The evolution of fungus-growing termites and their mutualistic fungal symbionts. *Proceedings of the National Academy of Sciences*, 99, 14887-14892.
DOI: https://doi.org/10.1073/pnas.222313099

Aoki, T., Kasson, M.T., Berger, M.C., Freeman, S., Geiser, D.M. & O'Donnell, K. (2018). *Fusarium oligoseptatum* sp. nov., a mycosymbiont of the ambrosia beetle *Euwallacea validus* in the Eastern US and typification of *F. ambrosium*. *Fungal Systematics and Evolution*, 1, 23-39.
DOI: https://doi.org/10.3114/fuse.2018.01.03

Aoki, T., Smith, J.A., Kasson, M.T., Freeman, S., Geiser, D.M., et al. (2019). Three novel Ambrosia *Fusarium* Clade species producing clavate macroconidia known (*F. floridanum* and *F. obliquiseptatum*) or predicted (*F. tuaranense*) to be farmed by *Euwallacea* spp. (Coleoptera: Scolytinae) on woody hosts. *Mycologia*, 111, 919-935.
DOI: https://doi.org/10.1080/00275514.2019.1647074

Ballard, R.G., Walsh, M.A. & Cole, W.E. (1982). Blue-stain fungi in xylem of lodgepole pine: A light-microscope study on extent of hyphal distribution. *Canadian Journal of Botany*, 60, 2334-2341.
DOI: https://doi.org/10.1139/b82-285

Ballard, R.G., Walsh, M.A. & Cole, W.E. (1984). The penetration and growth of blue-stain fungi in the sapwood of lodgepole pine attacked by mountain pine beetle. *Canadian Journal of Botany*, 62, 1724-1729.
DOI: https://doi.org/10.1139/b84-233

Bateman, C., Huang, Y.T., Simmons, D.R., Kasson, M.T., Stanley, E.L. & Hulcr, J. (2017). Ambrosia beetle *Premnobius cavipennis* (Scolytinae: Ipini) carries highly divergent ascomycotan ambrosia fungus, *Afroraffaelea ambrosiae* gen. nov. et sp. nov. (Ophiostomatales). *Fungal Ecology*, 25, 41-49.
DOI: https://doi.org/10.1016/j.funeco.2016.10.008

Batra, L.R. (1963). Ecology of ambrosia fungi and their dissemination by beetles. *Transactions of the Kansas Academy of Science*, 66, 213-236.
DOI: https://doi.org/10.2307/3626562

Batra, L.R. (1966). Ambrosia fungi: Extent of specificity to ambrosia beetles. *Science*, 153, 193-195.
DOI: https://doi.org/10.1126/science.153.3732.193

Batra, L.R. (1967). Ambrosia fungi: A taxonomic revision, and nutritional studies of some species. *Mycologia*, 59, 976-1017.
DOI: https://doi.org/10.1080/00275514.1967.12018485

Batra, L.R. & Michie, M.D. (1963). Pleomorphism in some ambrosia and related fungi. *Transactions of the Kansas Academy of Science*, 66, 470-481.
DOI: https://doi.org/10.2307/3626545

Beaver, R.A. (1986). The taxonomy, mycangia and biology of *Hypothenemus curtipennis* (Schedl), the first known cryphaline ambrosia beetle (Coleoptera: Scolytinae). *Insect Systematics & Evolution*, 17, 131-135.
DOI: https://doi.org/10.1163/187631286X00189

Boucher, D.H., James, S. & Keeler, K.H. (1982). The ecology of mutualism. *Annual Review of Ecology and Systematics*, 13, 315-347.
DOI: https://doi.org/10.1146/annurev.es.13.110182.001531

Bracewell, R.R. & Six, D.L. (2015). Experimental evidence of bark beetle adaptation to a fungal symbiont. *Ecology and Evolution*, 5, 5109-5119.
DOI: https://doi.org/10.1002/ece3.1772

Brader, L. (1964). Etude de la relation entre le scolyte des rameaux du cafeier, *Xyleborus compactus* Eichh. (*X. morstatti* Hag.), et sa plante-hote. Thesis, Veenman.

Branstetter, M.G., Ješovnik, A., Sosa-Calvo, J., Lloyd, M.W., Faircloth,B.C., et al. (2017). Dry habitats were crucibles of domestication in the evolution of agriculture in ants. *Proceedings of the Royal Society B: Biological Sciences*, 284, 20170095.
DOI: https://doi.org/10.1098/rspb.2017.0095

Brayford, D. (1987). *Fusarium bugnicourtii* sp. nov., and its relationship to *F. tumidum* and *F. tumidum* var. *coeruleum*. *Transactions of the British Mycological Society*, 89, 347-351.

Brockerhoff, E.G., Bain, J., Kimberley, M. & Knížek, M. (2006). Interception frequency of exotic bark and ambrosia beetles (Coleoptera: Scolytinae) and relationship with establishment in New Zealand and worldwide. *Canadian Journal of Forest Research*, 36, 289-298.
DOI: https://doi.org/10.1139/x05-250

Broennimann, O. & Guisan, A. (2008). Predicting current and future biological invasions: Both native and invaded ranges matter. *Biology Letters*, 4, 585-589.
DOI: https://doi.org/10.1098/rsbl.2008.0254

Butin, H., & Zimmermann, G. (1972). Zwei neue holzverfärbende *Ceratocystis*-Arten in Buchenholz (*Fagus sylvatica* L.). *Journal of Phytopathology*, 74, 281-287.
DOI: https://doi.org/10.1111/j.1439-0434.1972.tb02583.x

Carrillo, D., Duncan, R.E., & Peña, J.E. (2012). Ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) that breed in avocado wood in Florida. *Florida Entomologist*, 95, 573-579.
DOI: https://doi.org/10.1653/024.095.0306

Carrillo, D., Duncan, R.E., Ploetz, J.N., Campbell, A.F., Ploetz, R.C. & Peña, J.E. (2014). Lateral transfer of a phytopathogenic symbiont among native and exotic ambrosia beetles. *Plant Pathology*, 63, 54-62.
DOI: https://doi.org/10.1111/ppa.12073

Carrillo, J.D., Rugman-Jones, P.F., Husein, D., Stajich, J.E., Kasson, M.T., et al. (2019). Members of the *Euwallacea fornicatus* species complex exhibit promiscuous mutualism with ambrosia fungi in Taiwan. *Fungal Genetics and Biology*, 133, 103269.
DOI: https://doi.org/10.1016/j.fgb.2019.103269

Cassar, S. & Blackwell, M. (1996). Convergent origins of ambrosia fungi. *Mycologia*, 88, 596-601.
DOI: https://doi.org/10.1080/00275514.1996.12026690

Cognato, A.I., Hulcr, J., Dole, S.A. & Jordal, B.H. (2011). Phylogeny of haplo–diploid, fungus-growing ambrosia beetles (Curculionidae: Scolytinae: Xyleborini) inferred from molecular and morphological data. *Zoologica Scripta*, 40, 174-186.
DOI: https://doi.org/10.1111/j.1463-6409.2010.00466.x

Colunga-Garcia, M., Haack, R.A. & Adelaja, A.O. (2009). Freight transportation and the potential for invasions of exotic insects in urban and periurban forests of the United States. *Journal of Economic Entomology*, 102, 237-246.
DOI: https://doi.org/10.1603/029.102.0133

Cook, J.M. & Rasplus, J.Y. (2003). Mutualists with attitude: coevolving fig wasps and figs. *Trends in Ecology & Evolution*, 18, 241-248.
DOI: https://doi.org/10.1016/S0169-5347(03)00062-4

Cruz, L.F., Rocio, S., Duran, L., Menocal, O., Garcia-Avila, C. & Carrillo, D. (2018). Developmental biology of *Xyleborus bispinatus* (Coleoptera: Curculionidae) reared on an artificial medium and fungal cultivation of symbiotic fungi in the beetle's galleries. *Fungal Ecology*, 35, 116-126.
DOI: https://doi.org/10.1016/j.funeco.2018.07.007

Cruz, L.F., Menocal, O., Mantilla, J., Ibarra-Juarez, L.A. & Carrillo, D. (2019). *Xyleborus volvulus* (Coleoptera: Curculionidae): biology and fungal associates. *Applied and Environmental Microbiology*, 85, e01190-01119.
DOI: https://doi.org/10.1128/AEM.01190-19

Davidson, R.W. (1958). Additional species of Ophiostomataceae from Colorado. *Mycologia*, 50, 661-670.

Davidson, R.W. (1971). New species of *Ceratocystis*. *Mycologia*, 63, 5-15.

Davidson, R.W. (1979). A *Ceratocystis* associated with an ambrosia beetles in *Dendroctonus*-killed pines. *Mycologia*, 71, 1085-1089.

De Bary, A. (1879). *Die Erscheinung der Symbiose*. Verlag von Karl J. Trübner.
DOI: https://doi.org/10.1515/9783111471839-fm

De Beer, Z.W., Seifert, K.A. & Wingfield, M.J. (2013a). The ophiostomatoid fungi: their dual position in the Sordariomycetes. In: Seifert, K.A., De Beer, Z.W. & Wingfield, M.J. (Eds.), *The Ophiostomatoid Fungi: Expanding Frontiers*, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, pp. 12, 1-19.

De Beer, Z.W., Seifert, K.A. & Wingfield, M.J. (2013b). A nomenclator for Ophiostomatoid genera and species in the *Ophiostomatales* and *Microascales*. In: Seifert, K.A., De Beer, Z.W. & Wingfield, M.J. (Eds.), *The Ophiostomatoid Fungi: Expanding Frontiers*, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, pp. 245-322.

De Errasti, A., De Beer, Z.W., Coetzee, M., Roux, J., Rajchenberg, M., & Wingfield, M.J. (2016). Three new species of Ophiostomatales from *Nothofagus* in Patagonia. *Mycological Progress*, 15, 17.
DOI: 10.1007/s11557-016-1158-z

De Fine Licht, H.H. & Biedermann, P.H. (2012). Patterns of functional enzyme activity in fungus farming ambrosia beetles. *Frontiers in Zoology*, 9, 1-11.
DOI: https://doi.org/10.1186/1742-9994-9-13

Dreaden, T.J., Davis, J.M., De Beer, Z.W., Ploetz, R.C., Soltis, P.S., et al. (2014). Phylogeny of ambrosia beetle symbionts in the genus *Raffaelea*. *Fungal Biology*, 118, 970-978.
DOI: https://doi.org/10.1016/j.funbio.2014.09.001

Endoh, R., Suzuki, M., Okada, G., Takeuchi, Y. & Futai, K. (2011). Fungus symbionts colonizing the galleries of the ambrosia beetle *Platypus quercivorus*. *Microbial Ecology*, 62, 106-120.
DOI: https://doi.org/ 10.1007/s00248-011-9838-3

Eskalen, A., Gonzalez, A., Wang, D., Twizeyimana, M., Mayorquin, J., & Lynch, S. (2012). First report of a *Fusarium* sp. and its vector tea shot hole borer (*Euwallacea fornicatus*) causing *Fusarium* dieback on avocado in California. Plant Disease, 96(7), 1070-1070.
DOI: https://doi.org/10.1094/PDIS-03-12-0276-PDN

Eskalen, A., Stouthamer, R., Lynch, S.C., Rugman-Jones, P.F., Twizeyimana, M., et al. (2013). Host range of *Fusarium* dieback and its ambrosia beetle (Coleoptera: Scolytinae) vector in Southern California. *Plant Disease*, 97, 938-951.
DOI: https://doi.org/ 10.1094/PDIS-11-12-1026-RE

FABI (2021). The Polyphagous Shot Hole Borer (PSHB) and its fungus in South Africa. Accessed: 17-03-2021.
Link: https://www.fabinet.up.ac.za/index.php/pshb.

Fahrner, S. & Aukema, B.H. (2018). Correlates of spread rates for introduced insects. *Global Ecology and Biogeography*, 27, 734-743.
DOI: https://doi.org/10.1111/geb.12737

Farrell, B.D., Sequeira, A.S., O'Meara, B.C., Normark, B.B., Chung, J.H. & Jordal, B.H. (2001). The evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodinae). *Evolution*, 55, 2011-2027.

DOI: https://doi.org/10.1111/j.0014-3820.2001.tb01318.x

Farris, S. (1963). Ambrosia fungus storage in two species of *Gnathotrichus* Eichhoff (Coleoptera: Scolytinae). *The Canadian Entomologist*, 95, 257-259.
DOI: https://doi.org/10.4039/Ent95257-3

Fell S & De Beer WZ (2019). The impact of the Polyphagous Shot Hole Borer on Pecan trees in South Africa. *SA Pecan*, 82, 11-13.

Frank, A.B. (1877). Uber die biologischen verhaltnisse des thallus eineger krustenflechten. *Beitrage zur Biologie der Pflanzen*, 2S, 123-200.

Fraedrich, S.W., Harrington, T.C., Rabaglia, R.J., Ulyshen, M.D., Mayfield-Iii, A.E., et al. (2008). A fungal symbiont of the redbay ambrosia beetle causes a lethal wilt in redbay and other Lauraceae in the Southeastern United States. *Plant Disease*, 92, 215-224.
DOI: https://doi.org/ 10.1094/PDIS-92-2-0215

Freeman, S., Sharon, M., Maymon, M., Mendel, Z., Protasov, A., et al. (2013). *Fusarium euwallaceae* sp. nov.- a symbiotic fungus of *Euwallacea* sp., an invasive ambrosia beetle in Israel and California. *Mycologia*, 105, 1595-1606.
DOI: https://doi.org/10.3852/13-066

Freeman, S., Protasov, A., Sharon, M., Mohotti, K., Eliyahu, M., et al. (2012). Obligate feed requirement of *Fusarium* sp. nov., an avocado wilting agent, by the ambrosia beetle *Euwallacea aff. fornicata*. *Symbiosis*, 58, 245-251.
DOI: https://doi.org/10.1007/s13199-013-0222-6

Funk, A. (1970). Fungal symbionts of the ambrosia beetle *Gnathotrichus sulcatus. Canadian Journal of Botany*, 48, 1445-1448.
DOI: https://doi.org/10.1139/b70-217

Gadd, C.H. & Loos, C.A. (1947). The ambrosia fungus of *Xyleborus fornicatus* Eich. *Transactions of the British Mycological Society*, 31, 13-IN11.
DOI: https://doi.org/10.1016/S0007-1536(47)80003-8

Gebhardt, H., Kirschner, R., & Oberwinkler, F. (2002). A new *Ophiostoma* species isolated from the ambrosia beetle *Xyleborus dryographus* (Coleoptera: Scolytinae). *Mycological Progress*, 1, 377-382.
DOI: doi:10.1007/s11557-006-0034-7

Gebhardt, H., Begerow, D. & Oberwinkler, F. (2004). Identification of the ambrosia fungus of *Xyleborus monographus* and *X. dryographus* (Coleoptera: Curculionidae, Scolytinae). *Mycological Progress*, 3, 95-102.
DOI: https://doi.org/10.1007/s11557-006-0080-1

Gebhardt, H. & Oberwinkler, F. (2005). Conidial development in selected ambrosial species of the genus *Raffaelea*. *Antonie van Leeuwenhoek*, 88, 61-65.
DOI: https://doi.org/10.1007/s10482-004-7838-8

Gebhardt, H., Weiss, M. & Oberwinkler, F. (2005). *Dryadomyces amasae*: a nutritional fungus associated with ambrosia beetles of the genus *Amasa* (Coleoptera: Curculionidae, Scolytinae). *Mycological Research*, 109, 687-696.
DOI: https://doi.org/10.1017/S0953756205002777

Geiser, D.M., Al-Hatmi, A., Aoki, T., Arie, T., Balmas, V., et al. (2020). Phylogenomic analysis of a 55.1 kb 19-gene dataset resolves a monophyletic *Fusarium* that includes the *Fusarium solani* species complex. *Phytopathology*. (Online)
DOI: https://doi.ort/10.1094/PHYTO-08-20-0330-LE

Gibbs, J.N. (1993). The biology of ophiostomatoid fungi causing sapstain in trees and freshly cut logs. In: Wingfield, M.J., Seifert, K.A. & Webber, J.A. (Eds.), *Ceratocystis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity*, American Phytopathology Society, pp. 141–152.

Guerrero, R.T. (1966). Una nueva especie de hongo inperfecto asociado con el coleoptero *Platypus sulcatus* Chapius. *Revta Investnes Agropecuaria Series*, 5, 97-103.

Haack, R.A. (2001). Intercepted Scolytinae (Coleoptera) at US ports of entry: 1985–2000. *Integrated Pest Management Reviews*, 6, 253-282.
DOI: https://doi.org/10.1023/A:1025715200538

Haack, R.A. (2006). Exotic bark-and wood-boring Coleoptera in the United States: recent establishments and interceptions. *Canadian Journal of Forest Research*, 36, 269-288.
DOI: https://doi.org/10.1139/x05-249

Haanstad, J.O. & Norris, D.M. (1985). Microbial symbiotes of the ambrosia beetle *Xyloterinus politus*. *Microbial Ecology*, 11, 267-276.

Harrington, T.C. (2005). Ecology and evolution of mycophagous bark beetles and their fungal partners. In: Vega, F.E. & Blackwell, M. (Eds.), *Insect-Fungal Associations: Ecology and Evolution*, Oxford University Press, pp. 257-291.

Harrington, T.C., Fraedrich, S. & Aghayeva, D.N. (2008). *Raffaelea lauricola*, a new ambrosia beetle symbiont and pathogen on the Lauracea. *Mycotaxon*, 104, 399-404.
DOI: https://doi.org/10.3390/f8020048

Harrington, T.C., Aghayeva, D.N. & Fraedrich, S. (2010). New combinations in *Raffaelea, Ambrosiella*, and *Hyalorhinocladiella*, and four new species from the redbay ambrosia beetle, *Xyleborus glabratus*. *Mycotaxon*, 111, 337-361.
DOI: https://doi.org/10.5248/111.337

Harrington, T.C., McNew, D., Mayers, C., Fraedrich, S.W., & Reed, S.E. (2014). *Ambrosiella roeperi* sp. nov. is the mycangial symbiont of the granulate ambrosia beetle, *Xylosandrus crassiusculus*. *Mycologia*, 106, 835-845.
DOI: https://doi.org/10.3852/13-354

Hartig, T. (1844). Ambrosia des *Bostrichus dispar*. *Allg Forst-Jagdztg* 13, 73-74.

Hausner, G., Reid, J. & Klassen, G.R. (1993). On the phylogeny of *Ophiostoma*, *Ceratocystis* s.s., and *Microascus*, and the relationships within *Ophiostoma* based on partial ribosomal DNA sequences. *Canadian Journal of Botany*, 71, 1249-1265.
DOI: https://doi.org/10.1139/b93-148

Hsiau, P.T.W. & Harrington, T.C. (2003). Phylogenetics and adaptations of basidiomycetous fungi fed upon by bark beetles (Coleoptera: Scolytinae). *Symbiosis*, 34, 111-132.

Hubbard, H. (1896). Ambrosia beetles. *United States Department of Agriculture Yearbook* 1896, 421-430.

Hulcr, J. & Cognato, A.I. (2010). Repeated evolution of crop theft in fungus-farming ambrosia beetles. *Evolution: International Journal of Organic Evolution*, 64, 3205-3212.
DOI: https://doi.org/10.1111/j.1558-5646.2010.01055.x

Hulcr, J., Mann, R. & Stelinski, L.L. (2011). The scent of a partner: Ambrosia beetles are attracted to volatiles from their fungal symbionts. *Journal of Chemical Ecology*, 37, 1374-1377.
DOI: https://doi.org/10.1007/s10886-011-0046-x

Hulcr, J. & Stelinski, L.L. (2017). The ambrosia symbiosis: From evolutionary ecology to practical management. *Annual Review of Entomology*, 62, 285-303.
DOI: https://doi.org/10.1146/annurev-ento-031616-035105

Hulcr, J., Black, A., Prior, K., Chen, C.Y. & Li, H.F. (2017). Studies of ambrosia beetles (Coleoptera: Curculionidae) in their native ranges help predict invasion impact. *Florida Entomologist*, 100, 257-261.
DOI: https://doi.org/10.1653/024.100.0219

Ibarra-Juarez, L.A., Burton, M.A.J., Biedermann, P.H.W, Cruz, L., Desgarennes, D., et al. (2020) Evidence for succession and putative metabolic roles of fungi and bacteria in the farming mutualism of the ambrosia beetle *Xyleborus affinis*. *mSystems*, 5, e00541-00520.
DOI: https://doi.org/10.1128/mSystems.00541-20

Jankowiak, R., Bilański, P., Strzałka, B., Linnakoski, R., Bosak, A., & Hausner, G. (2019). Four new *Ophiostoma* species associated with conifer- and hardwood-infesting bark and ambrosia beetles from the Czech Republic and Poland. *Antonie van Leeuwenhoek*, 112, 1501-1521.
DOI: 10.1007/s10482-019-01277-5

Jankowiak, R., Ostafińska, A., Aas, T., Solheim, H., Bilański, P., et al. (2018). Three new *Leptographium* spp. (Ophiostomatales) infecting hardwood trees in Norway and Poland. *Antonie van Leeuwenhoek*, 111, 2323-2347.
DOI: 10.1007/s10482-018-1123-8

Jankowiak, R., Strzałka, B., Bilański, P., Linnakoski, R., Aas, T., et al. (2017). Two new *Leptographium* spp. reveal an emerging complex of hardwood-infecting species in the Ophiostomatales. *Antonie van Leeuwenhoek*, 110, 1537-1553.
DOI: https://doi.org/10.1007/s10482-017-0905-8

Jordal, B.H., Normark, B.B. & Farrell, B.D. (2000). Evolutionary radiation of an inbreeding haplodiploid beetle lineage (Curculionidae, Scolytinae). *Biological Journal of the Linnean Society* 71, 483-499.
DOI: https://doi.org/10.1111/j.1095-8312.2000.tb01270.x

Jordal, B.H. & Cognato, A.I. (2012). Molecular phylogeny of bark and ambrosia beetles reveals multiple origins of fungus farming during periods of global warming. *BMC Evolutionary Biology*, 12, 1-10.
DOI: https://doi.org/10.1186/1471-2148-12-133

Jordal, B.H. (2015). Molecular phylogeny and biogeography of the weevil subfamily Platypodinae reveals evolutionarily conserved range patterns. *Molecular Phylogenetics and Evolution* 92, 294-307.
DOI: https://doi.org/10.1016/j.ympev.2015.05.028

Kajii, C., Morita, T., Jikumaru, S., Kajimura, H., Yamaoka, Y. & Kuroda, K. (2013). Xylem dysfunction in *Ficus carica* infected with wilt fungus *Ceratocystis ficicola* and the role of the vector beetle *Euwallacea interjectus*. *IAWA Journal*, 34, 301-312.
DOI: https://doi.org/10.1163/22941932-00000025

Kajimura, H. & Hijii, N. (1992). Dynamics of the fungal symbionts in the gallery system and the mycangia of the ambrosia beetle, *Xylosandrus mutilatus* (Blandford) (Coleoptera: Scolytinae) in relation to its life history. *Ecological Research*, 7, 107-117.
DOI: https://doi.org/10.1007/BF02348489.

Kasson, M.T., O'Donnell, K., Rooney, A.P., Sink, S., Ploetz, R.C., et al. (2013). An inordinate fondness for *Fusarium*: Phylogenetic diversity of fusaria cultivated by ambrosia beetles in the genus *Euwallacea* on avocado and other plant hosts. *Fungal Genetics and Biology*, 56, 147-157.
DOI: https://doi.org/10.1016/j.fgb.2013.04.004

Kasson, M.T., Wickert, K.L., Stauder, C.M., Macias, A.M., Berger, M.C., et al. (2016). Mutualism with aggressive wood-degrading *Flavodon ambrosius* (Polyporales) facilitates niche expansion and communal social structure in *Ambrosiophilus* ambrosia beetles. *Fungal Ecology*, 23, 86-96.
DOI: https://doi.org/10.1016/j.funeco.2016.07.002

Kim, K.H., Choi, Y.J., Seo, S-T. & Shin, H-D. (2009). *Raffaelea quercus-mongolicae* sp. nov. associated with *Platypus koryoensis* on oak in Korea. *Mycotaxon* 110, 189-197.
DOI: https://doi.org/10.5248/110.189

Kinuura. H. (1995). Symbiotic fungi associated with ambrosia beetles. *Japan Agricultural Research Quarterly*, 29, 57-57.

Kirkendall, L.R. & Faccoli, M. (2010). Bark beetles and pinhole borers (Curculionidae, Scolytinae, Platypodinae) alien to Europe. *ZooKeys*, 227-251.
DOI: https://doi.org/10.3897/zookeys.56.529

Klepzig, K.D. & Six, D.L. (2004). Bark beetle-fungal symbiosis: Context dependency in complex associations. *Symbiosis*, 37, 189-205.

Klepzig, K.D., Adams, A.S., Handelsman, J. & Raffa, K.F. (2009). Symbioses: A key driver of insect physiological processes, ecological interactions, evolutionary diversification, and impacts on humans. *Environmental Entomology*, 38, 67-77.
DOI: https://doi.org/10.1603/022.038.0109

Kolařík, M. & Hulcr, J. (2009). Mycobiota associated with the ambrosia beetle *Scolytodes unipunctatus* (Coleoptera: Curculionidae, Scolytinae). *Mycological Research*, 113, 44-60.
DOI: https://doi.org/10.1016/j.mycres.2008.08.003

Kolařík, M. & Kirkendall, L.R. (2010). Evidence for a new lineage of primary ambrosia fungi in *Geosmithia* Pitt (Ascomycota: Hypocreales). *Fungal Biology*, 114, 676-689.
DOI: https://doi.org/10.1016/j.funbio.2010.06.005

Kolařík, M., Hulcr, J. & Kirkendall, L.R. (2015). New species of *Geosmithia* and *Graphium* associated with ambrosia beetles in Costa Rica. *Czech Mycology*, 67, 29-35.

Kostovcik, M., Bateman, C.C., Kolarik, M., Stelinski, L.L., Jordal, B.H. & Hulcr, J. (2015). The ambrosia symbiosis is specific in some species and promiscuous in others: evidence from community pyrosequencing. *The ISME Journal*, 9, 126-138.
DOI: https://doi.org/10.1038/ismej.2014.115

Krokene, P. & Solheim, H. (1998). Pathogenicity of four blue-stain fungi associated with aggressive and nonaggressive bark beetles. *Phytopathology*, 88, 39-44.
DOI: https://doi.org/10.1094/PHYTO.1998.88.1.39

Kubono, T. & Ito S. (2002). *Raffaelea quercivora* sp. nov. associated with mass mortality of Japanese oak, and the ambrosia beetle (*Platypus quercivorus*). *Mycoscience*, 43, 0255-0260.
DOI: https://doi.org/10.1007/S102670200037

Landi, L., Gómez, D.F., Braccini, C.L., Pereyra, V.A., Smith, S.M. & Marvaldi, A.E. (2017). Morphological and molecular identification of the invasive *Xylosandrus crassiusculus* (Coleoptera: Curculionidae: Scolytinae) and its South American range extending into Argentina and Uruguay. *Annals of the Entomological Society of America*, 110, 344-349.
DOI: https://doi.org/10.1093/aesa/sax032

Li, Y., Simmons, D.R., Bateman, C.C., Short, D.P., Kasson, M.T., et al. (2015). New fungus-insect symbiosis: culturing, molecular, and histological methods determine saprophytic Polyporales mutualists of *Ambrosiodmus* ambrosia beetles. *PLoS One*, 10, e0137689.
DOI: https://doi.org/10.1371/journal.pone.0137689

Li, Y., Bateman, C.C., Skelton, J., Jusino, M.A., Nolen, Z.J., et al. (2017). Wood decay fungus *Flavodon ambrosius* (Basidiomycota: Polyporales) is widely farmed by two genera of ambrosia beetles. *Fungal Biology*, 121, 984-989.
DOI: https://doi.org/10.1016/j.funbio.2017.08.004

Li, Y., Huang, Y.T., Kasson, M.T., Macias, A.M., Skelton, J., Carlson, P.S., et al. (2018). Specific and promiscuous ophiostomatalean fungi associated with Platypodinae ambrosia beetles in the Southeastern United States. *Fungal Ecology*, 35, 42-50.
DOI: https://doi.org/10.1016/j.funeco.2018.06.006

Liebhold, A.M., MacDonald, W.L., Bergdahl, D. & Mastro, V.C. (1995). Invasion by exotic forest pests: A threat to forest ecosystems. *Forest Science*, 41, a0001-z0001.

DOI: https://doi.org/10.1093/forestscience/41.s1.a0001

Lin, Y.T., Shih, H.H., Hulcr, J., Lin, C.S., Lu, S.S., & Chen, C.Y. (2017). *Ambrosiella* in Taiwan including one new species. *Mycoscience*, 58, 242-252.
DOI: https://doi.org/10.1016/j.myc.2017.02.004

Linnakoski, R., De Beer, Z.W., Ahtiainen, J., Sidorov, E., Niemelä, P., et al. (2010). *Ophiostoma* spp. associated with pine-and spruce-infesting bark beetles in Finland and Russia. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, 25, 72-93.
DOI: https://doi.org/10.3767/003158510X550845

Lynch, S.C., Twizeyimana, M., Mayorquin, J.S., Wang. D.H., Na, F., et al. (2016). Identification, pathogenicity and abundance of *Paracremonium pembeum* sp. nov. and *Graphium euwallaceae* sp. nov. - two newly discovered mycangial associates of the Polyphagous Shot Hole Borer (*Euwallacea* sp.) in California. *Mycologia*, 108, 313-329.
DOI: https://doi.org/10.3852/15-063

Lynn, K.M.T., Wingfield, M.J., Durán. A., Marincowitz, S., Oliveira, L.S.S., et al. (2020). *Euwallacea perbrevis* (Coleoptera: Curculionidae: Scolytinae), a confirmed pest on *Acacia crassicarpa* in Riau, Indonesia, and a new fungal symbiont; *Fusarium rekanum* sp. nov. *Antonie van Leeuwenhoek*, 113, 803-823.
DOI: https://doi.org/10.1007/s10482-020-01392-8

Lynn, K.M.T., Wingfield, M.J., Durán, A., Oliveira, L.S.S., De Beer, Z.W., & Barnes, I. (2021). Novel *Fusarium* mutualists of two *Euwallacea* species infesting *Acacia crassicarpa* in Indonesia. *Mycologia*, 1-23.
DOI: https://doi.org/10.1080/00275514.2021.1875708

Machingambi, N.M., Roux, J., Dreyer, L.L. & Roets, F. (2014). Bark and ambrosia beetles (Curculionidae: Scolytinae), their phoretic mites (Acari) and associated *Geosmithia* species (Ascomycota: Hypocreales) from *Virgilia* trees in South Africa. *Fungal Biology*, 118, 472-483.
DOI: https://doi.org/10.1016/j.funbio.2014.03.006

Marin, M., Preisig, O., Wingfield, B.D., Kirisits, T., Yamaoka, Y. & Wingfield, M.J. (2005). Phenotypic and DNA sequence data comparisons reveal three discrete species in the *Ceratocystis polonica* species complex. *Mycological Research*, 109, 1137-1148.
DOI: https://doi.org/10.1017/S095375620500362X

Marini, L., Haack, R.A., Rabaglia, R.J., Toffolo, E.P., Battisti, A. & Faccoli, M. (2011). Exploring associations between international trade and environmental factors with establishment patterns of exotic Scolytinae. *Biological Invasions* 13, 2275-2288.
DOI: https://doi.org/10.1007/s10530-011-0039-2

Martin, B.D. & Schwab, E. (2012). Symbiosis: "Living together" in chaos. *Studies in the History of Biology*, 4, 7-25.

Martin, B.D. & Schwab, E. (2013). Current usage of symbiosis and associated terminology. *International Journal of Biology*, 5, 32.
DOI: http://dx.doi.org/10.5539/ijb.v5n1p32

Massoumi-Alamouti, S., Tsui, C.K. & Breuil, C. (2009). Multigene phylogeny of filamentous ambrosia fungi associated with ambrosia and bark beetles. *Mycological Research*, 113, 822-835.
DOI: https://doi.org/10.1016/j.mycres.2009.03.003

Masuya, H., Kaneko, S., & Yamaoka, Y. (1998). A new *Ophiostoma* species isolated from Japanese oak infested by *Platypus quercivorus*. *Mycoscience*, 39, 347-350.
DOI: https://doi.org/10.1007/BF02464020.

Mayers, C.G., McNew, D.L., Harrington, T.C., Roeper, R.A., Fraedrich, S.W., et al. (2015). Three genera in the Ceratocystidaceae are the respective symbionts of three independent lineages of ambrosia beetles with large, complex mycangia. *Fungal Biology*, 119, 1075-1092.

DOI: https://doi.org/10.1016/j.funbio.2015.08.002

Mayers, C.G., Harrington, T.C., & Ranger, C.M. (2017). First report of a sexual state in an ambrosia fungus: *Ambrosiella cleistominuta* sp. nov. associated with the ambrosia beetle *Anisandrus maiche*. *Botany*, 95, 503-512.
DOI: https://doi.org/ 10.1139/cjb-2016-0297

Mayers, C.G., Bateman, C.C. & Harrington, T.C. (2018). New *Meredithiella* species from mycangia of *Corthylus* ambrosia beetles suggest genus-level coadaptation but not species-level coevolution. *Mycologia*, 110, 63-78.
DOI: https://doi.org/10.1080/00275514.2017.1400353

Mayers, C.G., Harrington, T.C., Masuya, H., Jordal, B.H., McNew, et al. (2020). Patterns of coevolution between ambrosia beetle mycangia and the Ceratocystidaceae, with five new fungal genera and seven new species. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, 44, 41-66.
DOI: https://doi.org/10.3767/persoonia.2020.44.02

Mayers, C.G., Harrington, T.C., McNew, D.L., Roeper, R.A., Biedermann, P.H.W., et al. (2020). Four mycangium types and four genera of ambrosia fungi suggest a complex history of fungus farming in the ambrosia beetle tribe Xyloterini. *Mycologia*, 112, 1104-1137.
DOI:10.1080/00275514.2020.1755209.

Mehdiabadi, N.J. & Schultz, T.R. (2010). Natural history and phylogeny of the fungus-farming ants (Hymenoptera: Formicidae: Myrmicinae: Attini). *Myrmecological News*, 13, 37-55.

Mendel, Z., Protasov, A., Sharon, M., Zveibil, A., Yehuda, S.B., et al. (2012). An Asian ambrosia beetle *Euwallacea fornicatus* and its novel symbiotic fungus *Fusarium* sp. pose a serious threat to the Israeli avocado industry. *Phytoparasitica*, 40, 235-238.
DOI: https://doi.org/ 10.1007/s12600-012-0223-7

Meurisse, N., Rassati, D., Hurley, B.P., Brockerhoff, E.G., & Haack, R.A. (2019). Common pathways by which non-native forest insects move internationally and domestically. *Journal of Pest Science*, 92, 13-27.
DOI: https://doi.org/10.1007/s10340-018-0990-0

Mueller, U.G., Schultz, T.R., Currie, C.R., Adams, R.M. & Malloch, D. (2001). The origin of the attine ant-fungus mutualism. *The Quarterly Review of Biology*, 76, 169-197.

Mueller, U.G. & Gerardo, N.M. (2002). Fungus-farming insects: Multiple origins and diverse evolutionary histories. *Proceedings of the National Academy of Sciences*, 99, 15247-15249.
DOI: https://doi.org/10.1073/pnas.242594799

Mueller, U.G., Gerardo, N.M., Aanen, D.K., Six, D.L. & Schultz, T.R. (2005). The evolution of agriculture in insects. *Annual Review of Ecology, Evolution, and Systematics*, 36, 563-595.
DOI: https://doi.org/10.1146/annurev.ecolsys.36.102003.152626

Musvuugwa, T., De Beer, Z.W., Duong, T.A., Dreyer, L.L., Oberlander, K.C. & Roets, F. (2015). New species of Ophiostomatales from Scolytinae and Platypodinae beetles in the Cape Floristic Region, including the discovery of the sexual state of *Raffaelea*. *Antonie van Leeuwenhoek*, 108, 933-950.
DOI: https://doi.org/10.1007/s10482-015-0547-7

Na, F., Carrillo, J.D., Mayorquin, J.S., Ndinga-Muniania, C., Stajich, J.E., et al. (2018). Two novel fungal symbionts *Fusarium kuroshium* sp. nov. and *Graphium kuroshium* sp. nov. of Kuroshio Shot Hole Borer (*Euwallacea sp. nr. fornicatus*) cause *Fusarium* dieback on woody host species in California. *Plant Disease*, 102, 1154-1164.
DOI: https://doi.org/10.1094/PDIS-07-17-1042-RE

Nakashima, T., Goto, C. & Iizuka, T. (1987). The primary and auxiliary ambrosia fungi isolated from the ambrosia beetles, *Scolytoplatypus shogun* Blandford (Coleoptera Scolytinae) and

Crossotarsus niponicus Blandford (Coleoptera Platypodinae). *Journal of the Faculty of Agriculture, Hokkaido University*, 63, 185-208.
DOI: http://hdl.handle.net/2115/13057

Nalim, F.A., Samuels, G.J., Wijesundera, R.L. & Geiser, D.M. (2011). New species from the *Fusarium solani* species complex derived from perithecia and soil in the Old World tropics. *Mycologia*, 103, 1302-1330.
DOI: https://doi.org/10.3852/10-307

Nel, W.J., Duong, T.A., Wingfield, B.D., Wingfield, M.J. & De Beer, Z.W. (2018). A new genus and species for the globally important, multihost root pathogen *Thielaviopsis basicola*. *Plant Pathology*, 67, 871-882.
DOI: https://doi.org/10.1111/ppa.12803

Nel, W.J., De Beer, Z.W., Wingfield, M.J. & Duong, T.A. (2020). The granulate ambrosia beetle, *Xylosandrus crassiusculus* (Coleoptera: Curculionidae, Scolytinae), and its fungal symbiont found in South Africa. *Zootaxa*, 4838, 427-435.
DOI: https://doi.org/10.11646/zootaxa.4838.3.7

Nirenberg, H. (1990). Recent advances in the taxonomy of *Fusarium*. *Studies in Mycology*, 32, 91-101.

Nobre, T., Rouland-Lefèvre, C. & Aanen, D.K. (2010). Comparative biology of fungus cultivation in termites and ants. In: Bignell, D.E., Roisin, Y. & Lo, N. (Eds.), *Biology of Termites: A Modern Synthesis*, Springer, pp. 193-210.

Nobre, T., Fernandes, C., Boomsma, J.J., Korb, J. & Aanen, D.K. (2011a). Farming termites determine the genetic population structure of *Termitomyces* fungal symbionts. *Molecular Ecology*, 20, 2023-2033.
DOI: https://doi.org/10.1111/j.1365-294X.2011.05064.x

Nobre, T., Koné, N.A., Konaté, S., Linsenmair, K.E. & Aanen, D.K. (2011b). Dating the fungus-growing termites' mutualism shows a mixture between ancient codiversification and recent symbiont dispersal across divergent hosts. *Molecular Ecology*, 20, 2619-2627.
DOI: https://doi.org/10.1111/j.1365-294X.2011.05090.x

Paap, T., De Beer, Z.W., Migliorini, D., Nel, W.J. & Wingfield, M.J. (2018). The Polyphagous Shot Hole Borer (PSHB) and its fungal symbiont *Fusarium euwallaceae*: A new invasion in South Africa. *Australasian Plant Pathology*, 47, 231-237.
DOI: https://doi.org/10.1007/s13313-018-0545-0

Paap, T., Wingfield, M.J., De Beer Z.W. & Roets, F. (2020). Lessons from a major pest invasion: The Polyphagous Shot Hole Borer in South Africa. *South African Journal of Science* 116, 1-4.
DOI: https://doi.org/10.17159/sajs.2020/8757

Parliament of the Republic of South Africa (2017). Plant Health (Phytosanitary) Bill.

Peer, K. & Taborsky, M. (2005). Outbreeding depression, but no inbreeding depression in haplodiploid ambrosia beetles with regular sibling mating. *Evolution*, 59, 317-323.
DOI: https://doi.org/10.1111/j.0014-3820.2005.tb00992.x

Ploetz, R.C., Hulcr, J., Wingfield, M.J. & De Beer, Z.W. (2013). Destructive tree diseases associated with ambrosia and bark beetles: Black swan events in tree pathology? *Plant Disease*, 97, 856-872.
DOI: https://doi.org/10.1094/PDIS-01-13-0056-FE

Potgieter, L.J., Douwes, E., Gaertner, M., Measey, J., Paap, T. & Richardson, D.M. (2020). Biological invasions in South Africa's urban ecosystems: Patterns, processes, impacts, and management. In: Van Wilgen, B.W., Measey J., Richardson, D.M., Wilson, J.R. & Zengeya, T.A. (Eds.), *Biological Invasions in South Africa*, Springer, pp. 275-309.
DOI: https://doi.org/10.1007/978-3-030-32394-3_11

Procter, M., Nel, W.J., Marincowitz, S., Crous, P., & Wingfield, M.J. (2020). A new species of *Raffaelea* from beetle-infested *Leucaena leucocephala. Fungal Systematics and Evolution*, 6, 305.
DOI: https://doi.org/ 10.3114/fuse.2020.06.16

Rabaglia, R.J., Dole, S.A. & Cognato, A.I. (2006). Review of American Xyleborina (Coleoptera: Curculionidae: Scolytinae) occurring north of Mexico, with an illustrated key. *Annals of the Entomological Society of America*, 99, 1034-1056.
DOI: https://doi.org/10.1603/0013-8746(2006)99[1034:ROAXCC]2.0.CO;2

Redfern, D.B., Stoakley, J.T., Steele, H. & Minter, D.W. (1987). Dieback and death of larch caused by *Ceratocystis laricicola* sp. nov. following attack by *Ips cembrae*. *Plant Pathology*, 36, 467-480.
DOI: https://doi.org/10.1111/j.1365-3059.1987.tb02264.x

Saucedo-Carabez, J., Ploetz, R.C., Konkol, J., Carrillo, D. & Gazis, R. (2018). Partnerships between ambrosia beetles and fungi: lineage-specific promiscuity among vectors of the laurel wilt pathogen, *Raffaelea lauricola*. *Microbial Ecology*, 76, 925-940.
DOI: https://doi.org/10.1007/s00248-018-1188-y

Schedl, K.E. (1955). XXVIII.—Contributions to the morphology and taxonomy of the Scolytoidea: 147. New records and new species of Scolytoidea from Africa. *Journal of Natural History*, 8, 211-220.

Schedl, K.E. (1960). On two new collections of African Scolytinae: 187. Contribution to the morphology and taxonomy of the Scolytoidea. *Journal of Natural History*, 3, 349-352.

Schedl, K.E. (1965). South African bark and timber beetles. *Journal of the Entomological Society of Southern Africa*, 28, 110-116.

Schedl, K.E. (1970). South African bark and timber beetles. *Annals of the Transvaal Museum*, 26, 177-182.

Schedl, K.E. (1975). South African bark and timber beetles, 3. Contribution to the morphology and taxonomy of the Scolytoidea. *Annals of the Transvaal Museum*, 29, 275-281.

Schedl, K.E. (1982). Scolytoidea (Coleoptera), mainly from South Africa. *Annals of the Transvaal Museum* 33, 277-286.

Schmidberger J (1836). Naturgeschichte des Apfelborkenk¨afers Apate dispar. *Beitr Obstbaumzucht Naturgeschichte Obstb¨aumen sch¨adlichen Insekten*, 4, 213-230.

Scott, D.B. & Du Toit, J.W. (1970). Three new *Raffaelea* species. *Transactions of the British Mycological Society*, 55, 181-186.
DOI: https://doi.org/10.1016/S0007-1536(70)80002-X

Simmons, D.R., Li, Y., Bateman, C.C. & Hulcr, J. (2016a). *Flavodon ambrosius* sp. nov., a basidiomycetous mycosymbiont of *Ambrosiodmus* ambrosia beetles. *Mycotaxon*, 131, 277-285.
DOI: https://doi.org/10.5248/131.277

Simmons, D.R., De Beer, Z.W., Huang, YT., Bateman, C., Campbell, A.S., et al. (2016b). New *Raffaelea* species (Ophiostomatales) from the USA and Taiwan associated with ambrosia beetles and plant hosts. *IMA Fungus*, 7, 265-273.
DOI: https://doi.org/10.5598/imafungus.2016.07.02.06

Six, D.L. (2003). Bark beetle-fungus symbioses. In: Bourtzis, K. & Miller T.A. (Eds.), *Insect Symbiosis*, Taylor & Francis Group, pp. 97-114.

Six, D.L., Stone, W.D., De Beer, Z.W. & Woolfolk, S.W. (2009). *Ambrosiella beaveri* sp. nov., associated with an exotic ambrosia beetle, *Xylosandrus multilatus* (Coleoptera: Curculionidae, Scolytinae), in Mississippi, USA. *Antonie van Leeuwenhoek*, 96, 17-29.
DOI: https://doi.org/10.1007/s10482-009-9331-x

Six, D.L. (2012). Ecological and evolutionary determinants of bark beetle—fungus symbioses. *Insects*, 3, 339-366.

DOI: https://doi.org/10.3390/insects3010339

Six DL (2019). A major symbiont shift supports a major niche shift in a clade of tree-killing bark beetles. *Ecological Entomology*, 45, 190-201.
DOI: https://doi.org/10.1111/een.12786

Skelton, J., Johnson, A.J., Jusino, M.A., Bateman, C.C., Li, Y. & Hulcr, J. (2019a). A selective fungal transport organ (mycangium) maintains coarse phylogenetic congruence between fungus-farming ambrosia beetles and their symbionts. *Proceedings of the Royal Society B*, 286, 20182127.
DOI: https://doi.org/10.1098/rspb.2018.2127

Skelton, J., Jusino, M.A., Carlson, P.M., Smith, K., Banik, M.T., et al. (2019b). Relationships among wood-boring beetles, fungi, and the decomposition of forest biomass. *Molecular Ecology*, 28, 4971-4986.
DOI: https://doi.org/10.1111/mec.15263

Skelton, J., Loyd, A., Smith, J.A., Blanchette, R.A., Held, B.W. & Hulcr, J. (2019c). Experimental evidence that fungal symbionts of beetles suppress wood decay by competing with decay fungi. *PeerJ*, e27676v1.
DOI: https://doi.org/10.7287/peerj.preprints.27676v1

Smith, S.M., Gomez, D.F., Beaver, R.A., Hulcr, J. & Cognato, A.I. (2019). Reassessment of the species in the *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) complex after the rediscovery of the "lost" type specimen. *Insects*, 10, 261.
DOI: https://doi.org/10.3390/insects10090261

Spatafora, J.W. & Blackwell, M. (1994). The polyphyletic origins of Ophiostomatoid fungi. *Mycological Research*, 98, 1-9.
DOI: https://doi.org/10.1016/S0953-7562(09)80327-4

Stouthamer, R., Rugman-Jones, P., Thu, P.Q., Eskalen, A., Thibault, T., et al. (2017). Tracing the origin of a cryptic invader: Phylogeography of the *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) species complex. *Agricultural and Forest Entomology*, 19, 366-375.
DOI: https://doi.org/10.1111/afe.12215

Takahashi, Y., Matsushita, N. & Hogetsu, T. (2010). Spatial distribution of *Raffaelea quercivora* in xylem of naturally infested and inoculated oak trees. *Phytopathology*, 100, 747-755.
DOI: https://doi.org/10.1094/PHYTO-100-8-0747

Talbot, P.H.B. (1977). The *Sirex-Amylostereum-Pinus* association. *Annual Review of Phytopathology*, 15, 41-54.
DOI: https://doi.org/10.1146/annurev.py.15.090177.000353

Umeda, C., Eskalen, A., & Paine, T.D. (2016). Polyphagous shot hole borer and *Fusarium* dieback in California. In T. D. Paine & F. Lieutier (Eds.), *Insects and Diseases of Mediterranean Forest Systems*, Cham: Springer, pp. 757-767.
DOI: https://doi.org/10.1007/978-3-319-24744-1_26

Valiev, A., Ogel, Z.B. & Klepzig, K.D. (2009). Analysis of cellulase and polyphenol oxidase production by southern pine beetle associated fungi. *Symbiosis*, 49, 37-42.
DOI: https://doi.org/10.1007/s13199-009-0007-0

Van Den Berg, N., du Toit, M., Morgan, S.W., Fourie, G., & De Beer, Z.W. (2019). First report of *Fusarium euwallaceae* causing necrotic lesions on *Persea americana* in South Africa. *Plant Disease*, 103, 1774.
DOI: https://doi.org/10.1094/PDIS-10-18-1818-PDN

Van Der Linde, J.A., Six, D.L., De Beer, Z.W., Wingfield, M.J. & Roux, J. (2016). Novel ophiostomatalean fungi from galleries of *Cyrtogenius africus* (Scolytinae) infesting dying *Euphorbia ingens*. *Antonie van Leeuwenhoek*, 109, 589-601.

DOI: https://doi/org/10.1007/s10482-016-0661-1

Van Der Walt, J.P. (1972). The yeast genus *Ambrosiozyma* gen. nov. (ascomycetes). *Mycopathologia et Mycologia Applicate*, 46, 305-315.
DOI: https://doi.org/10.1007/BF02052126

Van Wyk, M., Roux, J., Barnes, I., Wingfield, B.D., Chhetri, D.B., et al. (2004). *Ceratocystis bhutanensis* sp. nov., associated with the bark beetle *Ips schmutzenhoferi* on *Picea spinulosa* in Bhutan. *Studies in Mycology*, 50, 365-379.

Vanderpool, D., Bracewell, R.R. & McCutcheon, J.P. (2018). Know your farmer: Ancient origins and multiple independent domestications of ambrosia beetle fungal cultivars. *Molecular Ecology*, 27, 2077-2094.
DOI: https://doi.org/10.1111/mec.14394

Wingfield, M.J., Seifert, K.A. & Webber, J. (1993). *Ceratocystis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity*. American Phytopathology Society.

Wingfield, M.J., Brockerhoff, E.G., Wingfield, B.D. & Slippers, B. (2015). Planted forest health: The need for a global strategy. *Science*, 349, 832-836.
DOI: https://doi.org/ 10.1126/science.aac6674

Wood, S.L. & Bright, D.E. (1992). A catalog of Scolytinae and Platypodinae (Coleoptera), part 2: Taxonomic index. *The Great Basin Naturalist Memoirs*, 13, pp. 1–1553.

Yun, Y.H., Suh, D.Y., Yoo, H.D., Oh, M.H. & Kim, S.H. (2015). Yeast associated with the ambrosia beetle, *Platypus koryoensis*, the pest of oak trees in Korea. *Mycobiology*, 43, 458-466.
DOI: https://doi.org/10.5941/MYCO.2015.43.4.458

CHAPTER 2

Ophiostomatalean fungi associated with wood boring beetles in South Africa including two new species

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ORIGINAL PAPER

Ophiostomatalean fungi associated with wood boring beetles in South Africa including two new species

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Abstract Ambrosia beetles are small wood inhabiting members of the Curculionidae that have evolved obligate symbioses with fungi. The fungal symbionts concentrate nutrients from within infested trees into a usable form for their beetle partners, which then utilize the fungi as their primary source of nutrition. Ambrosia beetle species associate with one or more primary symbiotic fungal species, but they also vector auxiliary symbionts, which may provide the beetle with developmental or ecological advantages. In this study we isolated and identified ophiostomatalean fungi associated with ambrosia beetles occurring in a native forest area in South Africa. Using a modified Bambara beetle trap, living ambrosia beetle specimens were collected and their fungal symbionts isolated. Four beetle species, three Scolytinae and one Bostrichidae, were collected. Five species of ophiostomatalean fungi were isolated from the beetles and were identified using both morphological characters and DNA sequence data. One of these species, *Raffaelea sulphurea,* was recorded from South Africa for the first time and two novel species were described as *Ceratocystiopsis lunata* sp. nov. and *Raffaelea promiscua* sp. nov.

Keywords Bostrichidae · *Ceratocystiopsis* · Ophiostomatales · *Raffaelea* · Xyleborini

Introduction

Ambrosia beetles are small wood boring insects that reside in the true weevil family Curculionidae. Approximately 3500 species have been described in two sub-families (Platypodinae and Scolytinae), all of which have obligate associations with filamentous fungi (Six 2012; Jordal 2015). These fungi are primarily Ascomycota that are cultivated along the gallery walls and serve as the primary food source of the beetles and their growing broods (Batra 1966; Massoumi-Alamouti et al. 2009). While developing in the brood galleries, the beetles collect the spores of the fungal symbionts and store them within specially evolved structures known as mycangia (Klepzig and Six 2004). This not only maintains the association of the fungal symbionts within and between different generations of the beetles, but also provides the fungi with a consistent means of dispersal and introduction into a relatively competition-free environment in which they proliferate (Six 2012). In return, the fungi

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concentrate available nutrients from the host into a form usable for their beetle partners (De Fine Licht and Biedermann 2012).

The symbiotic fungi of bark and ambrosia beetles represent a polyphyletic assemblage of filamentous fungal genera, which have evolved convergent morphological traits that favour insect dispersal (Cassar and Blackwell 1996; Zipfel et al. 2006). Most fungal symbionts of ambrosia beetles reside in the orders Ophiostomatales and Microascales, although some species of Hypocreales and Basidiomycota have also been discovered (Kolařík and Kirkendall 2010; Kasson et al. 2013, 2016; Machingambi et al. 2014; Lynn et al. 2020). In the Ophiostomatales, there are three genera regarded as primary ambrosia beetle symbionts. These include *Affroraffaelea* (Bateman et al. 2017), *Aureovirgo* (van der Linde et al. 2016), and *Raffaelea* sensu lato (Dreaden et al. 2014). Most of the remaining genera in this order associate with bark beetles although some species, are associates of other arthropods such as mites or they occur in non-insect niches such as soil (De Beer and Wingfield 2013). Additionally, the family includes species such as *Hawksworthiomyces lignivorus*, which was originally isolated from decaying telephone poles (De Meyer et al. 2008), as well as a small number of species in the *Sporothrix schenckii* clade that are opportunistic human and animal pathogens (López-Romero et al. 2011).

It has been argued for a relatively long time that obligate insect-fungus mutualisms, such as the ambrosia symbioses, represented a one-on-one relationship (Hubbard 1896; Talbot 1977; Cook and Rasplus 2003). This hypothesis appeared to hold true for ambrosia beetles as they were typically found associated with a single, dominant primary symbiont. However, Batra (1966, 1967) opposed this view and a few recent studies have shown that ambrosia beetles can also associate with multiple secondary (or auxiliary) fungal species (Kolařík and Kirkendall 2010; Carrillo et al. 2019). In many cases, these auxiliary associates reside in the same orders as the primary symbionts, and in some cases the primary symbiont of one beetle species may serve as an auxiliary species of another (Batra 1966). However, unlike the primary fungal symbionts, the roles of auxiliary species remain unclear although various hypotheses have been proposed. These include (1) serving as a nutritional source during brood development and succession (Freeman et al. 2016); (2) enabling a beetle to adapt to a new host or environment (Carillo et al. 2014) and (3); increasing beetle fitness by reducing host tree defences and allowing colonization by the primary symbiont (Saucedo et al. 2018).

In many Southern hemisphere countries, including South Africa, ambrosia beetles and their associated fungi are poorly known. This is attributed to the fact that most of these beetles are regarded as harmless, secondary pests infesting stressed or dying trees (Huclr et al. 2017). However, with increasing globalization and the introduction of invasive pests and their associated pathogenic fungi, interest regarding ambrosia beetles in their native ranges and their potential to become economically significant has increased (Liebhold et al. 1995; Ploetz et al. 2013; Hulcr et al. 2017). This elevated interest, as well as the importance of these insects and their fungal associates, prompted the present study to investigate the diversity ophiostomatalean fungi associated with some commonly encountered ambrosia beetles in a native forest area of South Africa.

Materials and methods

Collection of beetles and isolation of fungi

Beetle specimens were obtained from direct field sampling. Field collections were carried out at two locations in Tzaneen, Limpopo Province, South Africa (23° 42′ 29.491″ S 30° 5′ 57.638″ E and 23° 44′ 29.491″ S 30° 11′ 15.417″) using a modified Bambara beetle trap (Hulcr and McCoy 2015). The modified trap contained a wire mesh insert (gap size of 0.5 mm) between the entrance and collection zone, preventing the insects from making contact with the ethanol lure. Traps were set out in the late afternoon with 90% ethanol and left over-night, after which live beetles were collected early the following morning. Beetles were placed on the surfaces of 65 mm Petri dishes and allowed to walk over the agar. These Petri dishes contained malt extract agar (MEA: 2% malt extract and 2% Difco® agar, Biolab, Midrand, South Africa) amended with streptomycin (0.04%, Sigma-Aldrich, Missouri, United States) to control growth of bacteria and cycloheximide (0.03%, Sigma-Aldrich) that is selective for species in the Ophiostomatales. The beetles were removed from the plates after 24 h and

transferred into individual cryotubes containing 90% ethanol and stored at − 20 °C for species-level identification.

Petri dishes were inspected regularly for fungal growth and ophiostomatalean isolates were purified by transferring hyphal tips to new MEA plates. Multiple isolates with culture morphologies resembling those of the ophiostomatalean fungi were obtained and used for morphological and DNA sequence-based characterisation. Pure cultures are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (Table 1) and representative isolates of novel taxa were also deposited in the culture collection (CBS) of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands.

Identification of beetles

Beetle specimens were examined using an automated Zeiss Discovery V12 dissection microscope (Zeiss, Oberkocken, Germany). Based on overall morphology, beetles were first sorted into groups before they were identified to species level using the key of Rabaglia et al. (2006). The ventral, lateral and dorsal aspects of specimens were examined, and photographic images were captured using a Zeiss Axiocam IcC5 (Zeiss, Oberkocken, Germany). Focus-stacked photographs were produced for the dorsal aspects using Helicon Focus v. 5 (HeliconSoft, Kharkiv, Ukraine) with up to 30 different images.

One of the beetle specimens from which fungal isolates were obtained had obscure morphological characteristics and the specimen was subjected to PCR amplification and sequencing of the ribosomal large subunit (28S) gene region. DNA was extracted using the Macherey Nagel NucleoSpin Tissue Kit (Macherey–Nagel, Dueren, Germany) from the dissected head of the beetle. DNA extraction was performed following the manufacturer's protocols, except for the final elution volume that was reduced to 60 µl. PCR amplification of the partial ribosomal large subunit (28S) was done using the primers 3665 and 4068 (Belshaw and Quicke 1997; Cognato 2013) in 25 µL reaction volumes as described by Cognato (2013). PCR products were treated with ExoSAP-IT™ PCR Product Clean-up Reagent (ThermoFisher Scientific, Massachusetts, United States). Sequencing reactions were carried out in both the forward and reverse directions using the same primers used in PCR using the BigDye® Terminator v3.1 cycle sequencing kit (ThermoFisher Scientific) with an annealing temperature of 55 °C. Sequencing PCR products were precipitated using the sodium acetate/ethanol method and submitted to the DNA sanger sequencing facility based at the University of Pretoria for analyses on ABI PRISM®3500 Genetic Analyzer (Applied Biosystems, California, United States). The software package Sequence Scanner v. 1.0 (https://sequence-scanner-software.software.informer.com/) was used for quality assessment and editing of the obtained sequencing reads. The consensus sequence was used in a BLASTN search against NCBI GenBank nr/nt database (www.ncbi.nlm.nih.gov/Genbank) to confirm putative morphological identification. The newly obtained sequence has been deposited in NCBI GenBank with the accession number MT355516.

Identification of fungal isolates

The obtained fungal isolates were grouped based on culture morphology and DNA extraction was carried out using lyophilized mycelium of representative isolates following the method of De Beer et al. (2014). PCR amplification was carried out for the partial 28S ribosomal large subunit (LSU) using the primers LR0R and LR5 (Vilgalys and Hester 1990), the internal transcribed spacer region (ITS) using primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) and the partial Beta-tubulin (*β-tubulin*) gene using the primers T10 (O'Donnel and Cigelnik 1997) and Bt2B (Glass and Donaldson 1995). PCR reactions were carried out as described by De Beer et al. (2014) in 25 µL reaction volumes and the annealing temperature was set at 55 °C. PCR products were treated with ExoSAP-IT™ PCR Product Clean-up Reagent (ThermoFisher Scientific). Sequencing reactions, precipitation and quality assessment was carried out as described above. Reactions were performed in both the forward and reverse direction using the same primers used in PCR and an annealing temperature of 55 °C was used for all three gene regions. Newly obtained sequences have been deposited in NCBI GenBank with the accession numbers provided in Table 1.

Obtained consensus sequences were used in a BLASTN search against NCBI GenBank nr/nt database for putative identification to genus level. Based

Table 1 Isolates obtained in this study

Species	Isolate number	Beetle host	Locality	Genbank accession numbers		
				LSU	ITS	*β-tubulin*
Ceratocystiopsis lunata	CMW 55897[a]	*Xs. crassiusculus*	Tzaneen, Limpopo	MW028141	MW028169	MW066754
	CMW 55898	*Xs. crassiusculus*	Tzaneen, Limpopo	MW028140	MW028170	MW066755
Ophiostoma palustre	CMW 56170	Bostrichid beetle	Tzaneen, Limpopo	MW028135	MW028164	
	CMW 54253	Bostrichid beetle	Tzaneen, Limpopo	MW028137	MW028165	
	CMW 54254	Bostrichid beetle	Tzaneen, Limpopo	MW028139	MW028166	
	CMW 54255	Bostrichid beetle	Tzaneen, Limpopo	MW028138	MW028167	
	CMW 56171	Bostrichid beetle	Tzaneen, Limpopo	MW028136	MW028168	
Raffaelea arxii	CMW 55893	*Xbo. affinis*	Tzaneen, Limpopo	MW028147	MW028171	
	CMW 55894	*Xbo. affinis*	Tzaneen, Limpopo	MW028148		
	CMW 55895	*Xbo. affinis*	Tzaneen, Limpopo	MW028149	MW028172	
	CMW 55896	*Xbo. affinis*	Tzaneen, Limpopo	MW028150		
	WN19.12.18	*Xbo. affinis*	Tzaneen, Limpopo	MW028151		
Raffaelea promiscua	CMW 55899[a]	*Xbi.s saxesenii*	Tzaneen, Limpopo	MW028144	MW028176	MW066750
	CMW 55900	*Xbi. saxesenii*	Tzaneen, Limpopo	MW028145	MW028177	MW066751
	CMW 55901	*Xbi. saxesenii*	Tzaneen, Limpopo	MW028142		
	CMW 55902	*Xbi. saxesenii*	Tzaneen, Limpopo	MW028143		
	CMW 56172	*Xbi. saxesenii*	Tzaneen, Limpopo	MW028146	MW028178	MW066752
Raffaelea sulphurea	CMW 55779	*Xbi. saxesenii*	Tzaneen, Limpopo	MW028156		
	CMW 55780	*Xbi. saxesenii*	Tzaneen, Limpopo	MW028157	MW28174	
	CMW 55784	*Xbi. saxesenii*	Tzaneen, Limpopo	MW028158		
	CMW 55788	*Xbi. saxesenii*	Tzaneen, Limpopo	MW028159		
	CMW 55789	*Xbi. saxesenii*	Tzaneen, Limpopo	MW028160		
	CMW 55790	*Xbi. saxesenii*	Tzaneen, Limpopo	MW028161		
	CMW 55792	*Xbi. saxesenii*	Tzaneen, Limpopo	MW028152		
	CMW 55793	*Xbi. saxesenii*	Tzaneen, Limpopo	MW028153	MW028173	
	CMW 55794	*Xbi. saxesenii*	Tzaneen, Limpopo	MW028154		
	CMW 55795	*Xbi. saxesenii*	Tzaneen, Limpopo	MW028155		
	CMW 55781	*Xbi. saxesenii*	Tzaneen, Limpopo			
	CMW 55782	*Xbi. saxesenii*	Tzaneen, Limpopo			
	CMW 55783	*Xbi. saxesenii*	Tzaneen, Limpopo			
	CMW 55785	*Xbi. saxesenii*	Tzaneen, Limpopo			
	CMW 55786	*Xbi. saxesenii*	Tzaneen, Limpopo			
	CMW 55787	*Xbi. saxesenii*	Tzaneen, Limpopo			
	CMW 55791	*Xbi. saxesenii*	Tzaneen, Limpopo			
	CMW 55903	*Xbi. saxesenii*	Tzaneen, Limpopo			
	CMW 55796	*Xbi. saxesenii*	Tzaneen, Limpopo			
	CMW 55,797	*Xbi. saxesenii*	Tzaneen, Limpopo			
	CMW 55,798	*Xbi. saxesenii*	Tzaneen, Limpopo			

[a]Type strains

on these identifications, various datasets were prepared and analysed for each genus separately. Datasets were prepared using MEGA v. 7.0.26 (Kumar et al. 2016) and alignments were done using the online version of MAFFT v. 7 (Katoh and Standley 2013) with default settings. Due to high variability within the

three ITS datasets, alignment was subjected to Gblocks v. 0.91b (Castresana 2000) analysis with the less stringent options to remove ambiguous aligned positions before being used for phylogenetic analyses.

Maximum parsimony analyses were performed using MEGA v. 7.0.26. Phylogenies were generated using Subtree-Pruning-Regrafting algorithm starting with 10 random initial trees. Alignment gaps and missing data were included. Confidence levels for the nodes were tested using 1000 bootstrap replicates.

Maximum likelihood analyses were performed using the software raxmlGUI v. 2 (Silvestro and Michalak 2012; Elder et al. 2019) following the General Time Reversible + GAMMA (GTR + G) nucleotide substitution model. Ten random ML searches followed by 1000 bootstrap replicates were performed.

Bayesian inference analyses were performed using MrBayes v. 3.2.5 (Ronquist and Huelsenbeck 2003). Phylogenies were generated following the Markov Chain Monte Carlo (MCMC) method using the GTR + G model. Ten parallel runs, each with four independent MCMC chains were conducted. Trees were sampled every 1000 generations for 10 million generations. Trees sampled in the burn-in phase (25% of trees sampled) were discarded and posterior probabilities were calculated from the remaining trees. Multi-locus phylogenies were constructed for *Ceratocystiopsis* and *Raffaelea* using the same methods described above on the combined LSU, ITS and βT datasets.

Morphological observations

Microscopic structures—Fungal isolates were examined using a Zeiss AxioScop 2 compound microscope with an affixed Zeiss Axiocam 105 color camera and a Swift M3602-3DGL light microscope with a built-in 3-megapixel digital camera. Specimens of actively growing cultures were mounted in 80% lactic acid (ThermoFisher Scientific) and examined using bright field and differential interference contrast microscopy. Fifty to 100 measurements were made for all characteristic morphological structures using Zen Blue v. 2.6 (Zeiss). Measurements were taken for conidia, conidiophores, conidiogenous cells, and some additional characteristics when present. Values are presented as minimum—maximum.

Colony growth and morphology—A growth study was conducted to determine optimal growth conditions for two putative new species. Two isolates of each lineage were used to determine growth rate and culture morphology. Agar plugs of 5 mm in diameter were excised from the edge of an actively growing culture and placed, mycelium side down, onto the centre of a 65 mm 2% MEA plate. Plates were incubated in the dark at a temperature range from 15 to 30 °C at 5 °C intervals. Three replicates were carried out for each isolate at each temperature. Isolates were maintained for 10 days after which mean colony diameter was determined. Colony colours were determined using the colour charts of Rayner (1970).

Results

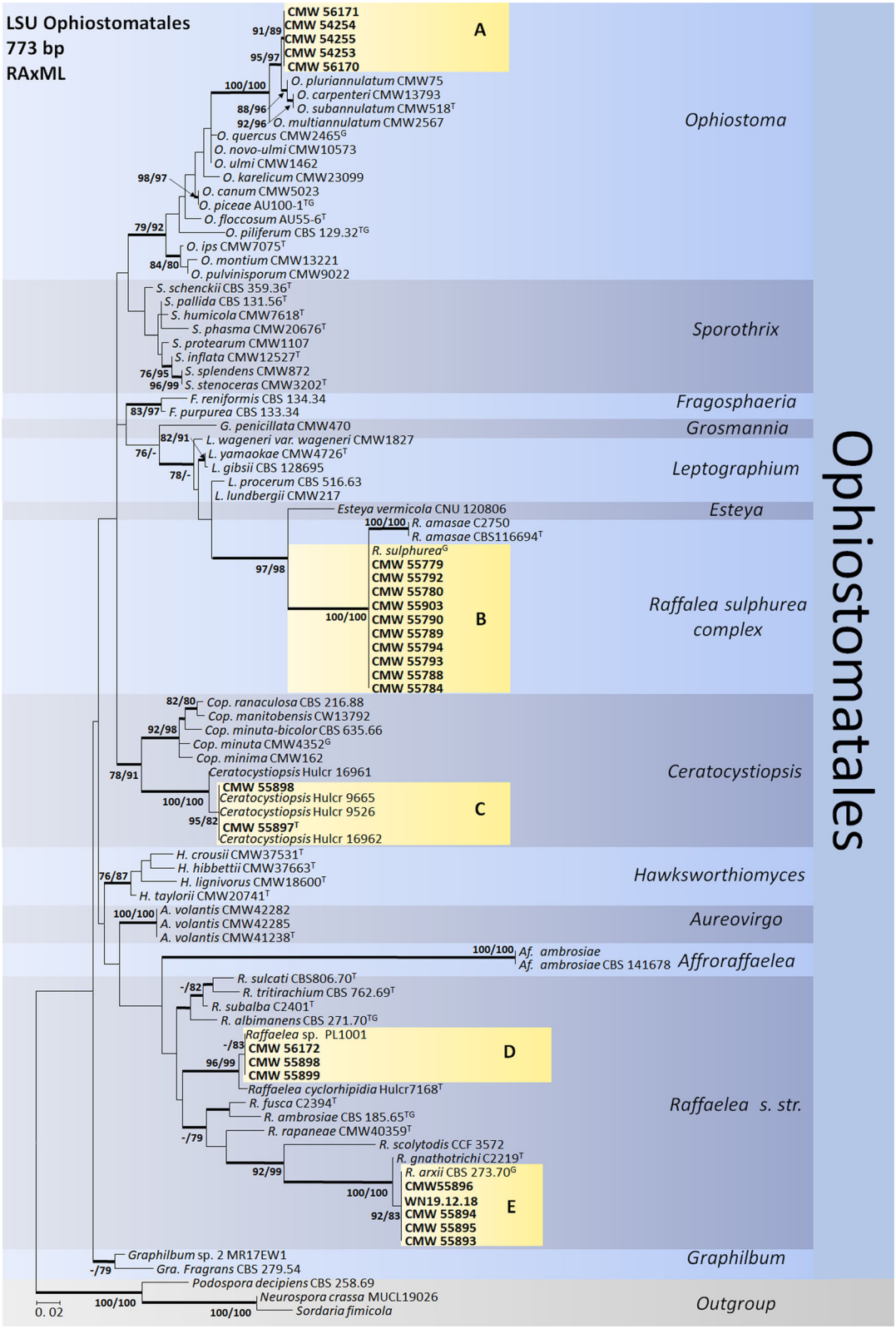
Identification of fungi

The collected beetle specimens yielded 38 isolates with morphologies resembling those of ophiostomatalean fungi. These included five distinct morphological groups and of these, 27 isolates (Table 1) were selected for DNA extraction, PCR amplification and sequencing of the LSU region.

BLAST searches using the LSU sequences revealed that all sequenced isolates were members of the Ophiostomatales. Based on the preliminary taxonomic groupings identified using BLAST, an LSU dataset (LSU-Ophiostomatales) was prepared and analysed to identify generic placement of the isolates. Phylogenetic analyses of the LSU-Ophiostomatales dataset (Fig. 1) including representative isolates of all genera of the Ophiostomatales, showed that the isolates considered in this study resided in in three different genera, *Ceratocystiopsis, Ophiostoma,* and *Raffaelea,* and represented five separate lineages (lineages A–E, Fig. 1).

Based on the genus and species complex assignments obtained from analyses of the LSU dataset (Fig. 1), seven genus level datasets were prepared and analysed (Tables 2, 3). The number of characters of each aligned dataset, numbers of parsimony informative characters, and additional information regarding phylogenetic analyses are presented in Table 3.

Ophiostoma—Based on the analyses of the LSU-Ophiostomatales dataset, five isolates grouped as a



◀**Fig. 1** RAxML phylogram derived from analysis of LSU data including all major groups in the Ophiostomatales. Bold branches indicate posterior probabilities ≥ 95%. Bootstrap values ≥ 75% for maximum likelihood and maximum parsimony analyses are indicated at nodes as ML/MP. G = sequence extracted from available genome. T = Ex-Type isolate

single lineage (A) in the *O. pluriannulatum* complex. Phylogenetic analyses of the ITS-*Oph* dataset (Fig. 2), that included a subset of *Ophiostoma* species focussed on the *O. pluriannulatum* complex, identified this species as *O. palustre*.

Ceratocystiopsis—Two isolates resided in the genus *Ceratocystiopsis* based on the analyses of the LSU-Ophiostomatales dataset. These isolates formed a distinct clade with high statistical support together with sequences of three undescribed isolates available from GenBank (lineage C). Phylogenetic analyses of the genus level datasets (LSU, ITS, and *β-tubulin*) (Fig. 3a-c) all supported this grouping. No ITS and *β-tubulin* sequence data were available for these undescribed isolates, but based on their grouping and sequence conservation in the LSU analysis, they likely belong to the same species as the isolates recovered from the current study.

Raffaelea—Twenty isolates, representing three morphological groups, grouped in three separate lineages within *Raffaelea* sensu lato (Fig. 1). Ten isolates grouped in the *Raffaelea sulphurea* complex (lineage B) and were identified as *R. sulphurea* based on phylogenetic analyses of LSU and ITS sequence data (Figs. 1 and 4a and b). The remaining ten isolates grouped as two distinct lineages (D and E) within *Raffaelea* sensu stricto (Figs. 1, 4). Five isolates were identified as *R. arxii* based on phylogenetic analyses of LSU and ITS sequence data (Figs. 1, 4a and b). The five remaining isolates (lineage D) together with an additional isolate labelled as *Raffaelea* sp. PL1001 (Eskalen & McDonald 2011; Dreaden et al. 2014), for which LSU and *β-tubulin* sequences are available in GenBank, formed a distinct lineage separate from all other species in the genus, but closely related to *R. cyclorhipidia* (Fig. 4b and c). An additional isolate labelled as *Raffaelea* sp. Hulcr7507 (Simmons et al. 2016), for which ITS and *β-tubulin* sequences are available on GenBank, grouped peripheral to this clade. Based on phylogenetic placement and ITS and *β-tubulin* sequence conservation this isolate most likely belongs to the same species as *Raffaelea* sp. PL1001 and the five isolates recovered from the current study.

Identification of beetles

The fungal isolates obtained in this study originated from 16 beetle specimens. Based on their morphological characters, these beetles were identified as four different species (Fig. 5). Three of these species resided in the Xyleborini (Scolytinae), namely *Xyleborinus (Xbi.) saxesenii; Xylosandrus (Xs.) crassiusculus;* and *Xyleborus (Xbo.) affinis* (Fig. 5b-d). The fourth was a species of Bostrichidae (Fig. 5a). Isolates used in this study originated from thirteen *Xbi. saxesenii* specimens, and a single specimen each of *Xs. crassiusculus, Xbo. affinis* and the unknown Bostrichid. Due to some obscured morphological characters for the *Xbo. affinis* specimen, DNA sequencing of the ribosomal large subunit was carried out and results positively confirmed our identification.

Beetle-fungus associations

Thirty-eight isolates with ophiostomatalean-like morphologies were isolated from 16 living beetle specimens. After initial separation based on morphology, 27 representative isolates were selected for further DNA sequence-based characterisation. Five of these fungal isolates were identified as *O. palustre* (Figs. 1, 2—lineage A) and all five isolates originated from a single beetle specimen in the Bostrichidae. Twenty-one isolates recovered from *Xbi. saxesenii* specimens were putatively identified as *R. sulphurea* based on morphology and this was confirmed by DNA sequencing of ten representative isolates originating from different beetles (Figs. 1,4—lineage B). Another five isolates also obtained from *Xbi. saxesenii* specimens were identified as a new species of *Raffaelea* (Figs. 1, 4—lineage D). Five isolates identified as *R. arxii* (Figs. 1, 4—lineage E) were obtained from a single beetle specimen identified as *Xbo. affinis*. Two isolates were obtained from a single specimen of *Xs. crassiusculus* and were identified as a new species of *Ceratocystiopsis* (Figs. 1, 3—lineage C).

Table 2 Isolates used in phylogenetic analyses and their sequence accession numbers

Species	Collection number	Genbank accession numbers		
		LSU	ITS	*β-tubulin*
Ceratocystiopsis brevicomis	CBS 137839	MW028162	MW028175	MW066757
C. brevicomis	UM1452	EU913683	EU913722	EU913761
C. collifera	CBS 126.89	EU913681	EU913721	–
C. concentrica	WIN(M) 71–07	AF135571	–	–
C. longisporum	UM48	EU913684	EU913723	–
C. manitobensis	UM237	EU913674	EU913714	EU913753
C. manitobensis	CBS 118838	DQ294358	–	–
C. minuta	CMW 4352	MW026163	MW028179	MW066756
C. minima	CBS 182.86	DQ294361	–	DQ296081
C. minima	UM1462	EU913663	EU913704	–
C. minuta-bicolor	CBS 635.66	MH870571	MH858901	EU977482
C. minuta-bicolor	CBS 393.77	DQ294359	–	DQ296079
C. pallidobrunnea	UM51	EU913682	–	–
C. ranaculosa	CBS 216.88	EU913673	EU913713	EU913752
C. ranaculosa	CBS 119683	DQ294357	–	DQ296077
C. rollhanseniana	CBS 118669	DQ294362	–	DQ296082
C. rollhanseniana	UM113	EU913678	EU913718	EU913757
Ceratocystiopsis sp.	Hulcr16961	LC363548	–	–
Ceratocystiopsis sp.	Hulcr9665	LC363540	–	–
Ceratocystiopsis sp.	Hulcr16962	LC363547	–	–
Ceratocystiopsis sp.	Hulcr9526	LC363538	–	–
Ophiostoma canum	CMW5023	DQ294372	–	–
O. carpenteri	CMW13793	DQ294363	–	–
O. flocossum	AU55-6[a]	AF234836	AF198231	–
O. himal-ulmi	C1183	–	AF198233	
O. ips	CMW7075[a]	DQ294381	AY546704	–
O. karelicum	CMW23099	EU443756	–	–
O. longiconidiatum	CMW17574[a]	–	EF408558	
O. montium	CMW13221	DQ294379	AY546711	–
O. multiannulatum	CMW2567[a]	DQ294366	FJ959049	–
O. novae-zelandiae	CIEFAP423	–	KT362249	
O. novo-ulmi	CMW10573	DQ294375	FJ430478	–
O. novo-ulmi s. americana	C510	–	AF198236	
O. palustre	CMW44403	–	KU865593	
O. piceae	AU100-1	AF234837	AF081129	LC090730
O. piliferum	CBS 129.32	DQ294377	–	AF221628
O. pluriannulatum	CMW75	DQ294365	AY934517	–
O. pulvinisporum	CMW9022[a]	DQ294380	AY546714	–
O. quercus	CMW2465	DQ294376	AY466626	AY466647
O. sparsiannulatum	CMW17231[a]	–	FJ906817	
O. subannulatum	CMW518[a]	DQ294364	AY934522	–
O. ulmi	CMW1462	DQ294374	AF198232	
Raffaelea aguacate	Raff. sp. 272	MT629748	MT633065	–

Table 2 continued

Species	Collection number	Genbank accession numbers		
		LSU	ITS	*β-tubulin*
R. albimanens	CBS 271.70[a]	MT629749	MT633066	MT644111
R. amasae	C2750[a]	MF399174	–	–
R. amasae	CBS116694[a]	EU984295	–	EU977470
R. ambrosiae	CBS 185.65[a]	MT629751	MT633067	MT644094
R. arxii	CBS 273.70	MT629754	MH859604	MW066753
R. brunnea	CBS 378.68[a]	EU984284	–	EU977460
R. campbellii	CMW44800[a]	KR018414	–	KX267112
R. canadensis	CBS 168.66[a]	EU984299	GQ225699	EU977473
R. crossotarsa	Hulcr7182[a]	KX267103	KX267135	KX267114
R. cyclorhipidia	Hulcr7168[a]	KX267104	KX267136	KX267115
R. ellipticospora	C2395	EU177446	–	KJ909298
R. fusca	C2394[a]	EU177449	–	KJ909301
R. gnathotrichi	C2219[a]	EU177460	–	–
R. lauricola	Raff. sp. 570	MT629759	MT633071	MT644093
R. montetyi	CBS 451.94[a]	EU984301	–	EU977475
R. quercivora	MAFF410918	MAFF410918	GQ225697	GQ225691
R. quercus-mongolicae	KACC44405	MT629763	MT633074	MT644091
R. rapaneae	CMW40359[a]	KT182935	KT192601	–
R. santoroi	CBS 399.67[a]	MH870707	MH859006	EU977476
R. scolytodis	CCF 3572	AM267270	–	–
R. subalba	C2401[a]	EU177443	–	KJ909305
R. subfusca	Hulcr4717	–	KX267137	KX267122
R. sulcati	CBS 806.70[a]	MH871752	–	EU977477
R. sulphurea	CBS 380.68	MT629768	MT633077	MT644092
R. tritiracium	CBS 762.69[a]	MH871169	MH859401	EU977478
R. xyleborina	Hulcr6099	KX267110	–	–
Raffaelea sp.	PL1001	KJ909293	–	KJ909295
Raffaelea sp.	Hulcr7507	–	KX267141	KX267128
Fragosphaeria purpurea	CBS 133.34	AF096191	–	–
F. reniformis	CBS 134.34	AB189155	–	–
Grosmannia penicillata	CMW470	DQ294385	–	–
Leptographium gibsii	CBS 128695	MH876512	–	–
L. lundbergii	CMW217	DQ294388	–	–
L. procerum	CBS 516.63	MH869960	–	–
L. yamaokae	CMW4726[a]	JN135315	–	–
L. wageneri var. wageneri	CMW1827	DQ294397	–	–
Esteya vermicola	CNU 120806	EU627684	–	–
Sporothrix humicola	CMW7618[a]	EF139114	–	–
S. inflata	CMW12527[a]	DQ294351	–	–
S. pallida	CBS 131.56[a]	EF139121	–	–
S. phasma	CMW20676[a]	DQ316151	–	–
S. protearum	CMW1107	DQ316145	–	–
S. splendens	CMW872	AF221013	–	–

Table 2 continued

Species	Collection number	Genbank accession numbers		
		LSU	ITS	*β-tubulin*
S. schenckii	CBS 359.36[a]	KX590890	–	–
S. stenoceras	CMW3202[a]	DQ294350	–	–
Hawksworthiomyces crousii	CMW37531[a]	KX396548	–	–
H. hibbettii	CMW37663[a]	KX396547	–	–
H. lignivorus	CMW18600[a]	EF139119	–	–
H. taylorii	CMW20741[a]	KX396546	–	–
Aureovirgo volantis	CMW42282	KR051133	–	–
Au. volantis	CMW42285	KR051134	–	–
Au. volantis	CMW41238[a]	KR051131	–	–
Affroraffaelea ambrosiae	n/a	KX620930	–	–
Af. ambrosiae	CBS 141678	NG057115	–	–
Graphilbum fragrans	CBS 279.54	MH868872	–	–
Graphilbum sp.2	MR17EW1	AY672929	–	–
Podospora decipiens	CBS 258.69	AY780073	–	–
Sordaria fimicola	n/a	AY545728	–	–
Neurospora crassa	MUCL19026	AF286411	–	–

[a]Type

Table 3 Number of characters strains and substitutional models used in phylogenetic analyses

	Dataset	LSU ophiostomatales	ITS *Oph*	LSU *Cer*	ITS *Cer*	βT *Cer*	LSU *Raf*	ITS *Raf*	βT *Raf*
Number of taxa		95	27	26	15	16	48	26	29
Number of characters	Total	496	567	690	565	283	530	403	473
	VPUC	13	34	26	63	11	29	24	56
	Constant	326	338	592	281	189	382	184	222
	PIC	157	195	72	221	83	119	195	195
MP	Tree length	649	408	159	633	178	341	770	1073
	CI	0.404	0.782	0.711	0.727	0.708	0.540	0.508	0.467
	RI	0.847	0.910	0.877	0.747	0.790	0.870	0.687	0.548

VPUC variable parsimony uninformative characters; *PIC* parsimony informative characters; *MP* maximum parsimony; *CI* consistency index; *RI* retention index

Taxonomy

Ceratocystiopsis lunata W.J. Nel sp. nov. Figure 6.

MycoBank MB838616 *Etymology*: Name reflects the crescent shaped conidia.

Description: Conidiophores mononematous, macronematous, arising from vegetative hyphae, simple, upright, straight, curved or undulate, 5–97 µm long (avg. 26.1 ± 15.8 µm). Conidiogenous cells integrated, hyaline, blastic, sometimes denticulate, 3–42 × 1.5–3.1 µm (avg. 13.7 ± 5.5 × 2.1 ± 0.3 µm). Conidia hyaline, aseptate, two types falcate to crescent shaped, no sheath 4.9–9.5 × 1.3–2.8 µm (avg. 6.6 ± 0.8 × 1.9 ± 0.3 µm) and oblong with the upper part swollen, apex round, tapering toward base,

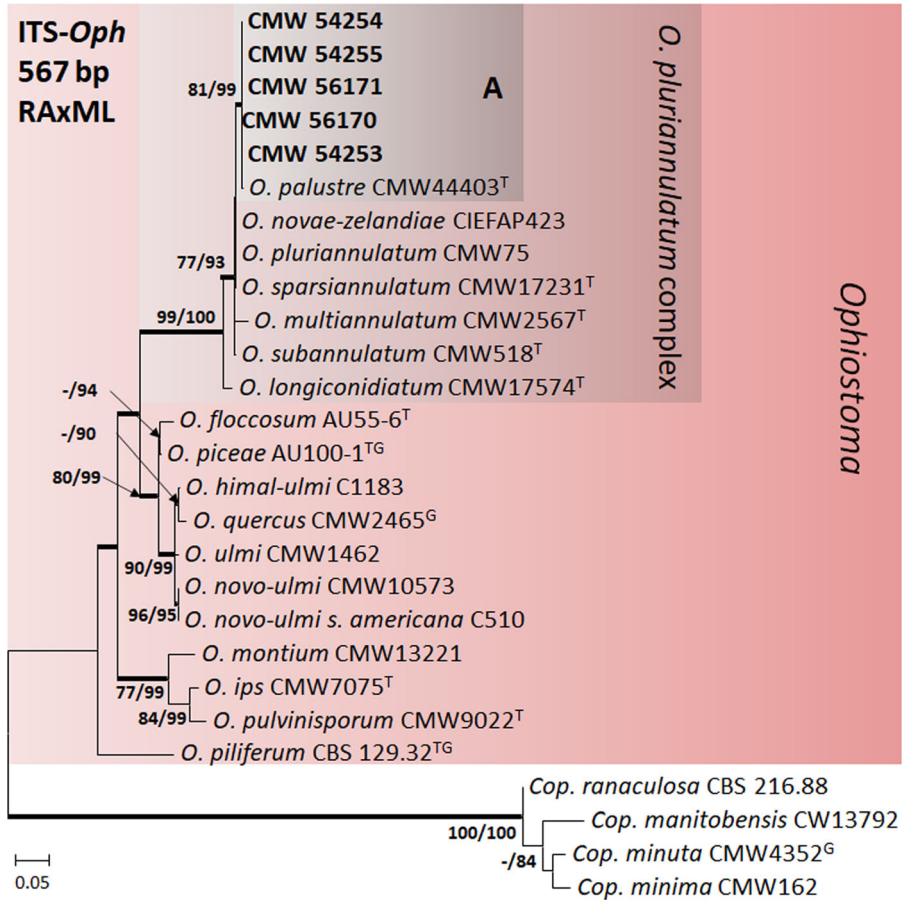


Fig. 2 RAxML phylogram derived from analysis of ITS-*Ophiostoma* data including taxa from the genus *Ophiostoma* specifically focussing on the *O. pluriannulatum* complex. Bold branches indicate posterior probabilities ≥ 95%. Bootstrap values ≥ 75% for maximum likelihood and maximum parsimony analyses are indicated at nodes as ML/MP. G = sequence extracted from available genome. T = Ex-Type isolate

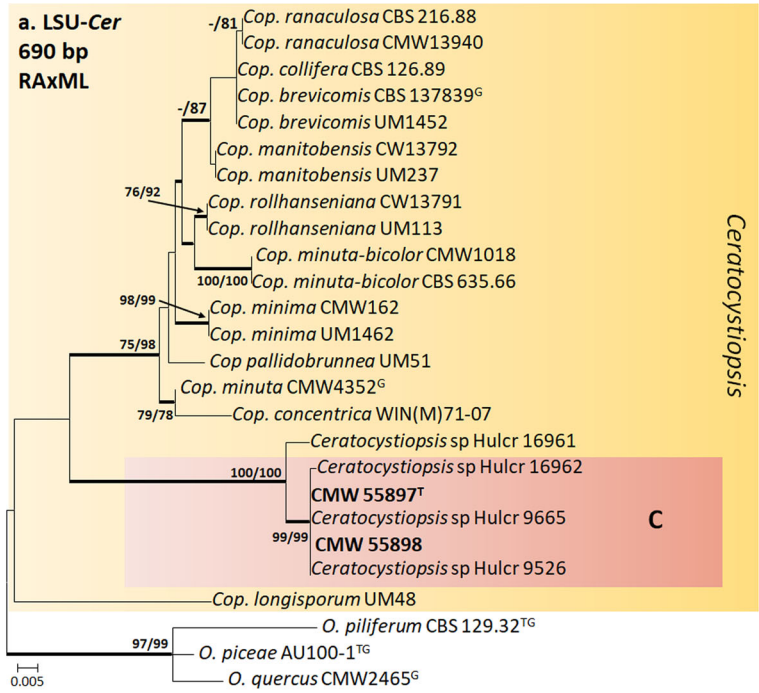
base truncated, 2–6 × 1.2–3.1 µm (avg. 4.5 ± 0.6 × 2.1 ± 0.4 µm), yeast-like budding observed in fresh culture.

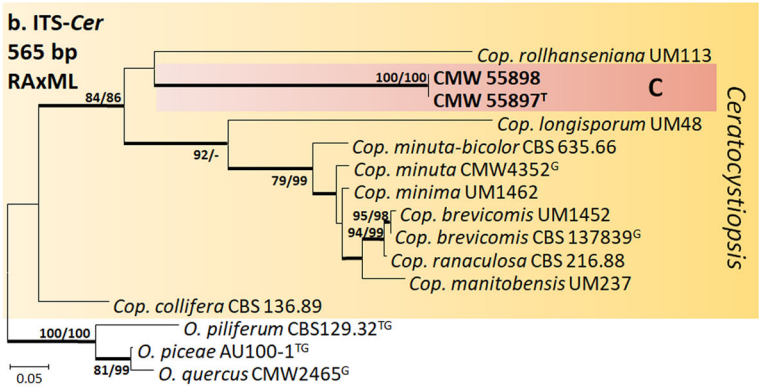
Specimens examined: South Africa, Limpopo, Tzaneen, isolated from a living *Xs. crassciusculus* beetle, W. J. Nel. 21 September 2019, holotype (PREM 63099, living culture ex-holotype CMW 55897 = CBS 147171).

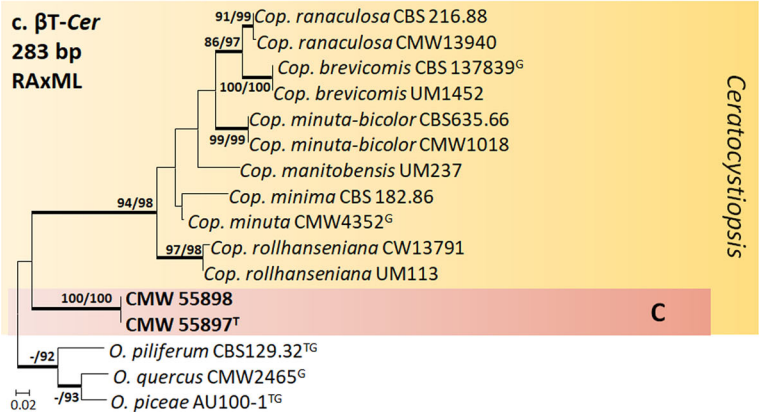
Additional specimens: South Africa, Limpopo, Tzaneen, isolated from a living *Xs. crassciusculus* beetle, W. J. Nel. 21 September 2019, paratype (PREM 63100, living culture CMW 55898 = CBS 147172).

Cultures: Moderate growth rate on 2% MEA in dark. Grows best at 30 °C reaching an average of 48.8 mm (± 0.9 mm) in 10 d. Colony growth circular with smooth margins, both abundant aerial and submerged mycelia present, flat, whitish to creamy in colour.

Notes: *Ceratocystiopsis lunata* can be distinguished from other species of *Ceratocystiopsis* based on its conidial morphology. *Ceratocystiopsis lunata* produces both falcate and oblong conidia whereas other species of *Ceratocystiopsis* typically only produce oblong conidia (De Beer and Wingfield 2013).







◀**Fig. 3** RAxML phylogenies derived from analyses of various *Ceratocystiopsis* datasets including all major taxa described in the genus. **a** Phylogram derived from the LSU analyses; **b** Phylogram derived from the ITS analyses; **c** Phylogram derived from the *β-tubulin* analyses. Bold branches indicate posterior probabilities ≥ 95%. Bootstrap values ≥ 75% for maximum likelihood and maximum parsimony analyses are indicated at nodes as ML/MP. G = sequence extracted from available genome. T = Ex-Type isolate

Raffaelea promiscua W.J. Nel sp. nov. Figure 7.

MycoBank MB838615 Etymology: Name refers to the promiscuous (*Promiscuum* L.) nature of the species that is associated with different ambrosia beetles.

Description: Conidiophores mononematous, macronematous, arising from vegetative hyphae, mostly simple, occasionally branched, upright, straight, curved or undulate, tapering towards apex, 14–195 µm long, reduced to conidiogenous cell. Conidiogenous cells integrated, hyaline to lightly pigmented, cylindrical, or peg-like, tapering towards apex, blastic, 5–56 × 1–3.9 µm (avg. 21.9 × 2.4 µm). Conidia hyaline, aseptate, majority oblong with the upper part swollen, apex round, tapering toward base, base truncated, 2–9 × 1–4 µm (avg. 5.1 × 2.5 µm), yeast-like budding observed in fresh culture.

Specimens examined: South Africa, Limpopo, Tzaneen, isolated from a living *Xbi. saxesenii* beetle, W. J. Nel. 21 September 2019, holotype (PREM 63101, living culture CMW 55899 = CBS 147173).

Additional specimens: South Africa, Limpopo, Tzaneen, isolated from a living *Xbi. saxesenii* beetle, W. J. Nel. 21 September 2019, paratype (PREM 63102, living culture CMW 55900 = CBS 147174). South Africa, Limpopo, Tzaneen, isolated from a living *Xbi. saxesenii* beetle, W. J. Nel. 21 September 2019 (living culture CMW 55901 = CBS 147175). South Africa, Limpopo, Tzaneen, isolated from a living *Xbi. saxesenii* beetle, W. J. Nel. 21 September 2019 (living culture CMW 55902 = CBS 147176).

Cultures: Slow growing on 2% MEA in dark. Grows best at 25 °C reaching and average of 34 mm (± 1.9 mm) in 10 d. Colonies circular with smooth margins, mycelia mostly submerged, aerial hyphae present, flat, initially whitish turning brownish olive to dark greyish brown with age starting at the centre of the colony. Yeast-like growth often present at inoculation site from initial growth of colony.

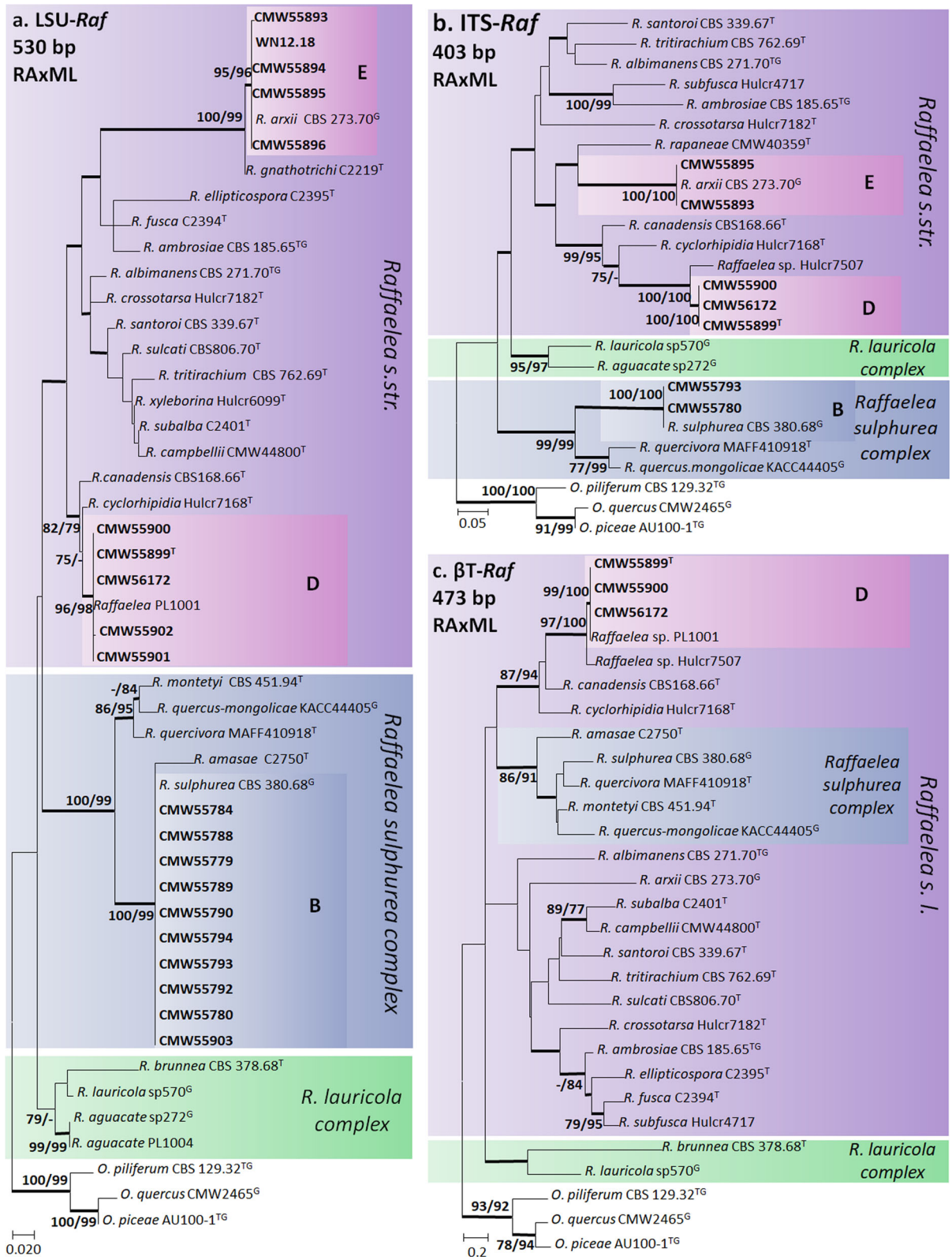
Notes: *Raffaelea promiscua* can be distinguished from its sister taxon *R. cyclorhipidia* (Simmons et al. 2016) by its smaller conidia and the fact that these are predominantly oblong with enlarged apices, and are 5.1 × 2.5 µm on average, whereas those of *R. cyclorhipidia* are elliptical to elongate and 7.3 × 3.5 µm on average. *Raffaelea promiscua* colonies are smooth with aerial hyphae whereas *R. cyclorhipidia* has a tough and wrinkled appearance.

Discussion

A total of 38 ophiostomatalean fungal isolates were obtained from 16 adult beetles representing *Xs. crassiusculus, Xbi. saxesenii, Xbo. affinis*, and an unidentified species of Bostrichidae. The fungi were identified as five distinct species in the Ophiostomatales, one of which was recorded from South Africa for the first time and two represented novel species described here as *C. lunata* and *R. promiscua*.

An unusual association of *O. palustre* with a species of Bostrichidae emerged from this study. Although some small species of Bostrichidae can easily be confused with species of Scolytinae (Ivie 2002), these beetles are not known to associate with fungi, preferentially infesting wood with a low moisture content (Creffield 1996; Ivie 2002). However, some species of *Ophiostoma,* including many species in the *O. pluriannulatum* complex such as *O. palustre* (Osorio et al. 2016), are associated with wounds on trees. It is consequently possible that the bostricid beetle accidentally picked up spores of *O. palustre* colonizing the wounded tissue induced by its gallery. Many ophiostomatalean fungi also associate with phoretic mites vectored between hosts by various beetles (Hofstetter et al. 2013) and it is also possible that the fungus originated from a mite carried by the beetle.

Xyleborinus saxesenii is an ambrosia beetle with a cosmopolitan distribution and is among the most



◀**Fig. 4** RAxML phylogenies derived from analyses of various *Raffaelea* datasets including all major taxa described in the genus. **a** Phylogram derived from the LSU analyses; **b** Phylogram derived from the ITS analyses; **c** Phylogram derived from the *β-tubulin* analyses. Bold branches indicate posterior probabilities ≥ 95%. Bootstrap values ≥ 75% for maximum likelihood and maximum parsimony analyses are indicated at nodes as ML/MP. G = sequence extracted from available genome. T = Ex-Type isolate

common ambrosia beetles found globally. This species was originally described from Germany (Ratzeburg 1837) and has been detected on every continent except Antarctica (Wood and Bright 1992). Considerable research has been conducted on the fungal associates of *Xbi. saxesenii*, with its primary fungal symbiont *Raffaelea sulphurea* first described by Batra (1967). Subsequently, a second dominant mycangial symbiont of this insect, *Fusicolla acetilerea*, was detected by Biedermann et al. (2013). *Xbi. saxesenii* is also associated with a number of less dominant fungi including species of *Paecilomyces, Cladosporium, Ramularia,* and *Aureobasidium* (Biedermann et al. 2013; Malacrinò et al. 2017).

Xyleborinus saxesenii was first reported in South Africa by Schedl (Schedl 1975) where it was collected in the Western Cape and Kwazulu-Natal provinces, but its fungal associates were not considered. In the present study, 26 fungal isolates were obtained from specimens of *Xbi. saxesenii*. Of these, 21 were identified as *R. sulphurea* and this represents the first report of the fungus from South Africa. The remaining five fungal isolates grouped together with an undescribed *Raffaelea* sp. isolated from an unknown ambrosia beetle recorded as PL1001 by Eskalen and McDonald (2011). Subsequent to its first report, this undescribed fungus has been identified in several studies associated with various Xyleborini including *Xbo. bispinatus, Xbo. volvulus* and *Xbi. saxesenii* (Cruz et al. 2018, 2019; Saucedo-Carabez et al. 2018). Based on their distinct morphology and phylogenetic grouping separating them from their closest relative, *R. cylcorhipidia,* the isolates from the present study, including *Raffaelea* sp. PL1001, were described as *R. promiscua.*

Xylosandrus crassiusculus is a commonly encountered, cosmopolitain ambrosia beetle. Yet relatively little is known regarding the fungi associated with this

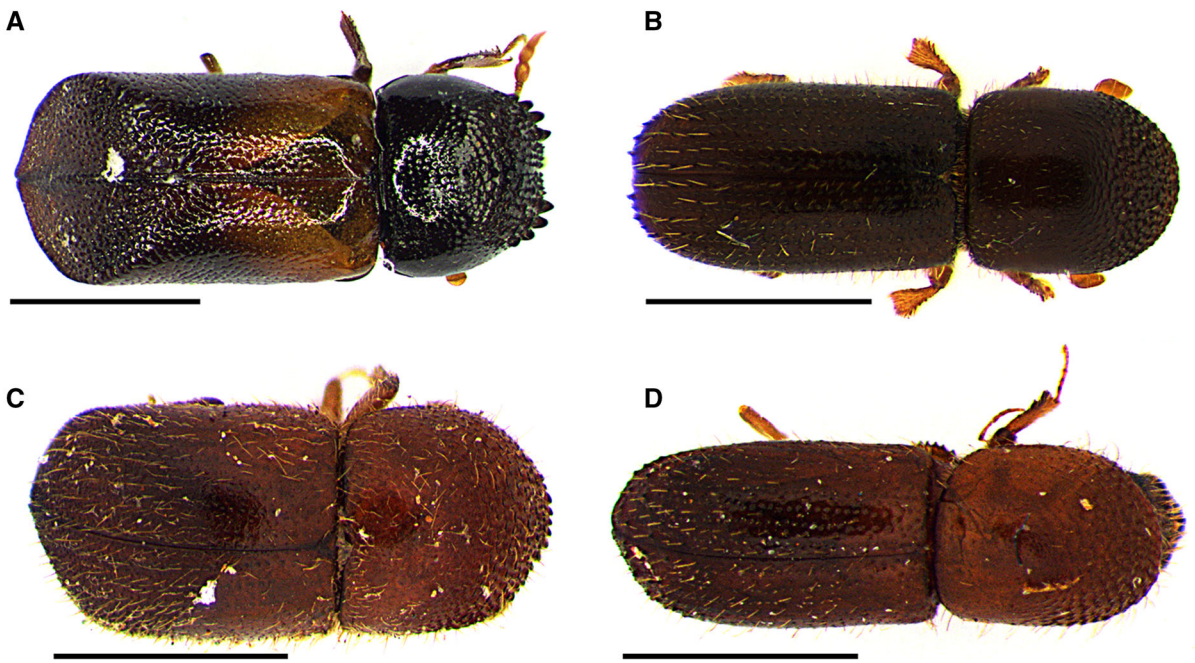


Fig. 5 Beetle species from which fungal isolates were obtained; **a** A unknown species of Bostrichidae beetle; **b** *Xyleborinus saxesenii*; **c** *Xylosandrus crassiusculus*; **d** *Xyleborus affinis*. Scale = 1 mm

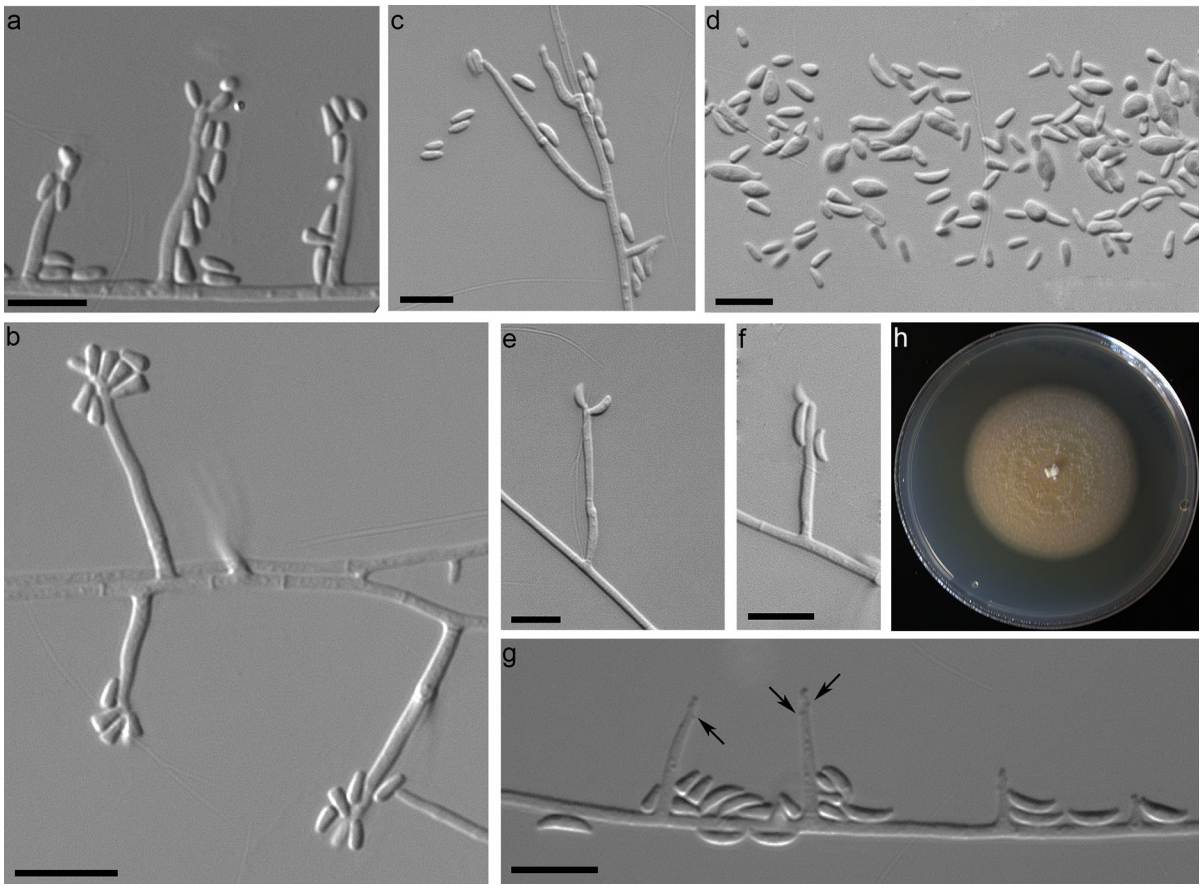


Fig. 6 Morphological characteristics of asexual structures of *Ceratocystiopsis lunata* sp. nov. **a–c** Conidiogenous cells giving rise to oblong conidia; **d** Conidia; **e–g** Conidiogenous cells giving rise to falcate conidia; **g** arrows Presence of denticles; **h** Pure culture gown on MEA in the dark for 12 days. Scale = 10 µm

beetle. Its primary fungal symbiont, *Ambrosiella roeperi*, was described relatively recently (Harrington et al. 2014), which is surprising given that the beetle was described as long ago as 1866. Recent pyrosequencing of the mycangial community of *Xs. crassiusculus* revealed that this niche is dominated by *Ambrosiella*, but other species including *Ceratocystis, Fusarium, Cladosporium* and various yeasts were also shown to be present (Kostovic et al. 2015).

In this study, two isolates of *Ceratocystiopsis* were obtained from a living *Xs. crassiusculus* specimen. Species of *Ceratocystiopsis* are common symbionts of scolytine bark beetles, which are close relatives of the ambrosia beetles. Previous studies investigating the fungal symbionts of ambrosia beetles in the Platypodinae showed that some species of *Ceratocystiopsis* have a promiscuous relationship with these insects (Inácio et al. 2012; Li et al. 2018). Li et al. (2018) concluded that their *Ceratocystiopsis* sp. 2 obtained from *Euplatypus compositus*, *E. parelellus* and *Oxoplatypus quadridentatus*, was the same species as *Ophiostoma* sp. X obtained from *Platypus cylindrus* in a study by Inácio et al. (2012). Phylogenetic analysis of the LSU region of the *Ceratocytiopsis* isolates obtained from *Xs. crassiusculus*in the present study, grouped these isolates along with those obtained by Li et al. (2018) and they were described here as *C. lunata*. This is the first time that *C. lunata* has been obtained from an ambrosia beetle in the Scolytinae. Our results, therefore, suggest that it could be an auxiliary symbiont of multiple species of ambrosia beetles.

Xyleborus affinis is a pan topical species of ambrosia beetle, native to tropical America (Rabgalia et al. 2006). This species has been introduced into Europe, Asia, Australia and Africa, where its presence was

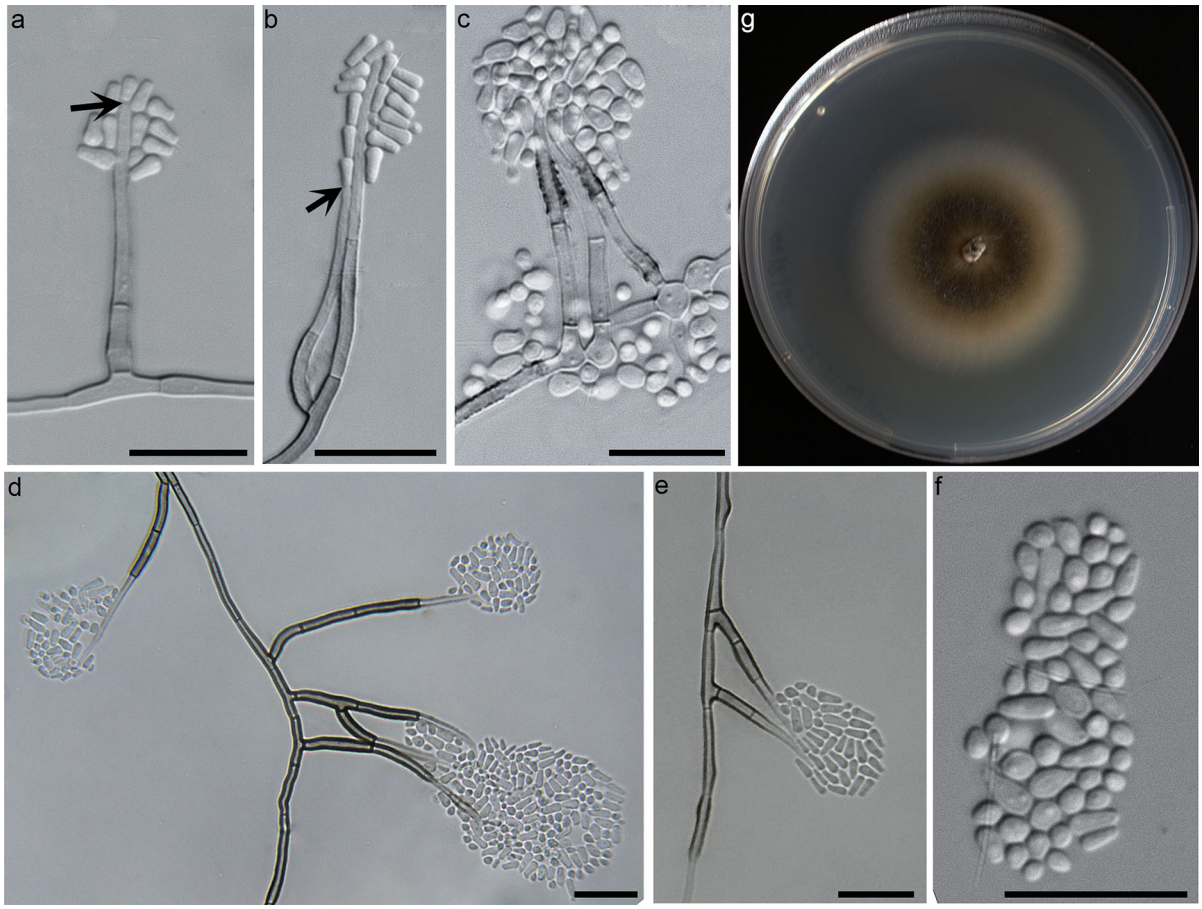


Fig. 7 Morphological characteristics of asexual structures of *Raffaelea promiscua* sp. nov. **a–e** Conidiogenous cells giving rise to conidia; **a** and **b** arrows indicate conidiogenesis taking place; **f** Conidia; **g** Pure culture gown on MEA in the dark for 12 days. Scale: **a–c**, **f** = 10 µm; **d–e** = 20 µm

later recorded in South Africa in the early 1980's (Schedl 1982; Rabgalia et al. 2006). Although the beetle was described more than a decade ago, its primary fungal symbiont is unknown. However, community pyrosequencing of the mycangia from 38 *Xbo. affinis* beetles showed that their fungal communities are highly diverse, including species from the Ophiostomatales, Microascales and a large variety of yeasts (Kostovic et al. 2015).

In this study, five isolates of *Raffaelea arxii* were obtained from a living specimen of *Xbo. affinis. Raffaelea arxii* was first described from South Africa as the primary fungal symbiont of *Xbo. torquatus* (= *Xbo. volvulus*) by Scott and Du Toit (1970). Aside from being the primary fungal symbiont of *Xbo. torquatus*, previous studies have found this species to be vectored by numerous other *Xyleborus* spp. including *Xbo. affinis* (Campbell et al. 2016; Saucedo-Carabez et al. 2018). Our findings provide additional support for *R. arxii* being the primary symbiont of both *Xbo. volvulus* and *Xbo. affinis,* as has previously been hypothesized (Saucedo et al. 2016). However, because *R. arxii* was obtained only from the body surfaces of the beetle specimen and not the mycangia, its symbiotic relationship with *Xbo. affinis* could not be deduced from this study.

Conclusions

Ambrosia beetles associate with various fungal symbionts that act as their source of nutrition, aid in their development and play an important part in their adaptive success. In this study, five species of

ophiostomatalean fungi were obtained from four species of wood-boring beetle, three species of scolytine ambrosia beetle and one species of bostrichid beetle. Based on morphological characters and DNA sequence data, two new species of ophiostomatalean fungi were described and one is reported from the country for the first time. This study, like many others investigating the ophiostomatalean fungi in South Africa has led to the discovery and description of new species, suggesting that there are more novel species to be discovered. However, with very few studies focussed on investigating the diversity of ophiostomatalean fungi associated with ambrosia beetles in South Africa, this appears to be a niche that warrants further investigation.

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Data availability All sequence data produced in the study have been made publicly available. All cultures have been deposited in accessible culture collections. Data sets generated and analysed in this study are available from the corresponding author upon request.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethics approval Approval obtained for study from institution. Registration number: NAS282/2019.

References

Bateman C, Huang Y-T, Simmons DR, Kasson MT, Stanley EL, Hulcr J (2017) Ambrosia beetle *Premnobius cavipennis* (*Scolytinae*: Ipini) carries highly divergent ascomycotan ambrosia fungus, *Afroraffaelea ambrosiae* gen. nov. et sp. nov. (Ophiostomatales). Fungal Ecol 25:41–49. https://doi.org/10.1016/j.funeco.2016.10.008

Batra LR (1966) Ambrosia fungi: extent of specificity to ambrosia beetles. Science 153:193–195. https://doi.org/10.1126/science.153.3732.193

Batra LR (1967) Ambrosia fungi: a taxonomic revision, and nutritional studies of some species. Mycologia 59:976–1017. https://doi.org/10.1080/00275514.1967.12018485

Belshaw R, Quicke DLJ (1997) A molecular phylogeny of the Aphidiinae (Hymenoptera: *Braconidae*). Mol Phylogenet Evol 7:281–293. https://doi.org/10.1006/mpev.1996.0400

Biedermann PH, Klepzig KD, Taborsky M, Six DL (2013) Abundance and dynamics of filamentous fungi in the complex ambrosia gardens of the primitively eusocial beetle *Xyleborinus saxesenii* Ratzeburg (*Coleoptera*: *Curculionidae*, *Scolytinae*). FEMS Microbiol 83:711–723. https://doi.org/10.1111/1574-6941.12026

Campbell AS, Ploetz RC, Dreaden TJ, Kendra PE, Montgomery WS (2016) Geographic variation in mycangial communities of *Xyleborus glabratus*. Mycologia 108:657–667. https://doi.org/10.3852/15-133

Carrillo D, Duncan R, Ploetz J, Campbell A, Ploetz R, Peña J (2014) Lateral transfer of a phytopathogenic symbiont among native and exotic ambrosia beetles. Plant Pathol 63:54–62. https://doi.org/10.1111/ppa.12073

Carrillo JD, Rugman-Jones PF, Husein D, Stajich JE, Kasson MT, Carrillo D, Stouthamer R, Eskalen A (2019) Members of the *Euwallacea fornicatus* species complex exhibit promiscuous mutualism with ambrosia fungi in Taiwan. Fungal Genet Biol 133:1–14. https://doi.org/10.1016/j.fgb.2019.103269

Cassar S, Blackwell M (1996) Convergent origins of ambrosia fungi. Mycologia 88:596–601. https://doi.org/10.1080/00275514.1996.12026690

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol and Evol 17:540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Cognato A (2013) Molecular phylogeny and taxonomic review of Premnobiini Browne, 1962 (*Coleoptera*: *Curculionidae*: *Scolytinae*). Front Ecol Evol. https://doi.org/10.3389/fevo.2013.00001

Cook JM, Rasplus J-Y (2003) Mutualists with attitude: coevolving fig wasps and figs. Trends Ecol Evol 18:241–248. https://doi.org/10.1016/S0169-5347(03)00062-4

Creffield JW (1996) Wood destroying insects: wood borers and termites. CSIRO publishing

Cruz LF, Menocal O, Mantilla J, Ibarra-Juarez LA, Carrillo D (2019) *Xyleborus volvulus* (*Coleoptera*: *Curculionidae*): biology and fungal associates. Appl Environ Microbiol 85:1–11. https://doi.org/10.1128/AEM.01190-19

Cruz LF, Rocio S, Duran L, Menocal O, Garcia-Avila C, Carrillo D (2018) Developmental biology of *Xyleborus bispinatus* (*Coleoptera*: *Curculionidae*) reared on an artificial medium and fungal cultivation of symbiotic fungi in the beetle's galleries. Fungal Ecol 35:116–126. https://doi.org/10.1016/j.funeco.2018.07.007

De Beer ZW, Duong TA, Barnes I, Wingfield BD, Wingfield MJ (2014) Redefining *Ceratocystis* and allied genera. Stud Mycol 79:187–219. https://doi.org/10.1016/j.simyco.2014.10.001

De Beer ZW, Wingfield MJ (2013) Emerging lineages in the ophiostomatales. In: Seifert KA, De Beer ZW, Wingfield MJ (eds) The ophiostomatoid fungi: expanding frontiers.

CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, pp 21–46

De Fine Licht HH, Biedermann PH (2012) Patterns of functional enzyme activity in fungus farming ambrosia beetles. Front Zoo 9:13. https://doi.org/10.1186/1742-9994-9-13

De Meyer EM, De Beer ZW, Summerbell RC, Moharram A, De Hoog GS, Vismer HF, Wingfield MJ (2008) Taxonomy and phylogeny of new wood-and soil-inhabiting *Sporothrix* species in the *Ophiostoma stenoceras-Sporothrix schenckii* complex. Mycologia 100:647–661. https://doi.org/10.3852/07-157R

Dreaden TJ, Davis JM, De Beer ZW, Ploetz RC, Soltis SP, Wingfield MJ, Smith JA (2014) Phylogeny of ambrosia beetle symbionts in the genus *Raffaelea*. Fungal Biol 118:970–978. https://doi.org/10.1016/j.funbio.2014.09.001

Edler D, Klein J, Antonelli A, Silvestro D (2019) raxmlgui20 beta: a graphical interface and toolkit for phylogenetic analyses using RAxML. bioRxiv. https://doi.org/10.1101/800912

Eskalen A, McDonald V (2011) First report of *Raffaelea canadensis* causing laurel wilt disease symptoms on avocado in California. Plant Dis 95:9–1189. https://doi.org/10.1094/PDIS-03-11-0203

Freeman S, Sharon M, Dori-Bachash M, Maymon M, Belausov E, Maoz Y, Margalit O, Protasov A, Mendel Z (2016) Symbiotic association of three fungal species throughout the life cycle of the ambrosia beetle *Euwallacea* nr. *fornicatus*. Symbiosis 68:115–128. https://doi.org/10.1007/s13199-015-0356-9

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61:1323–1330

Harrington TC, McNew D, Mayers C, Fraedrich SW, Reed SE (2014) *Ambrosiella roeperi* sp. nov. is the mycangial symbiont of the granulate ambrosia *beetle Xylosandrus crassiusculus*. Mycologia 106:835–845. https://doi.org/10.3852/13-354

Hofstetter RW, Moser J, Blomquist S (2013) Mites associated with bark beetles and their hyperphoretic ophiostomatoid fungi. In: Seifert KA, De Beer ZW, Wingfield MJ (eds) The ophiostomatoid fungi: expanding frontiers. CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, pp 165–176

Hubbard H (1896) Ambrosia beetles. United States Department of Agriculture Yearbook 1896:421–430

Hulcr J, Black A, Prior K, Chen C-Y, Li H-F (2017) Studies of ambrosia beetles (*Coleoptera*: *Curculionidae*) in their native ranges help predict invasion impact. Fla Entomol 100:257–261. https://doi.org/10.1653/024.100.0219

Hulcr J, McCoy N (2015) Catching beetles. http://www.ambrosiasymbiosis.org/ambrosia-beetles/catching-beetles/

Inácio ML, Henriques J, Nóbrega F, Marcelino J, Sousa E (2012) Ophiostomatoid fungi, a new threat to cork oak stands. Conference paper: present and future of cork oak in portugal in portugal held by the portuguese society of plant physiology, p 87–92

Ivie MA (2002) Bostrichidae latreille 1802. In: Arnett RHJr (†), Thomas MC, Skelley PE, Frank JH (ed) American beetles, Vol. II: *Polyphaga*: *Scarabaeoidea* through *Curculionoidea*, CRC Press, Boca Raton, London, New York, Washington, pp 233–244

Jordal BH (2015) Molecular phylogeny and biogeography of the weevil subfamily *Platypodinae* reveals evolutionarily conserved range patterns. Mol Phylogenet Evol 92:294–307. https://doi.org/10.1016/j.ympev.2015.05.028

Kasson MT, O'Donnell K, Rooney AP, Sink S, Ploetz RC, Ploetz JN, Konkol JL, Carrillo D, Freeman S, Mendel Z (2013) An inordinate fondness for *Fusarium*: phylogenetic diversity of fusaria cultivated by ambrosia beetles in the genus *Euwallacea* on avocado and other plant hosts. Fungal Genet Biol 56:147–157. https://doi.org/10.1016/j.fgb.2013.04.004

Kasson MT, Wickert KL, Stauder CM, Macias AM, Berger MC, Simmons DR, Short DP, DeVallance DB, Hulcr J (2016) Mutualism with aggressive wood-degrading *Flavodon ambrosius* (*Polyporales*) facilitates niche expansion and communal social structure in *Ambrosiophilus* ambrosia beetles. Fung Ecol 23:86–96. https://doi.org/10.1016/j.funeco.2016.07.002

Katoh K, Standley D (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol and Evol 30:772–780. https://doi.org/10.1093/molbev/mst010

Klepzig KD, Six D (2004) Bark beetle-fungal symbiosis: context dependency in complex associations. Symbiosis 3:189–205

Kolařík M, Kirkendall LR (2010) Evidence for a new lineage of primary ambrosia fungi in *Geosmithia* Pitt (*Ascomycota*: *Hypocreales*). Fungal Biol 114:676–689. https://doi.org/10.1016/j.funbio.2010.06.005

Kostovcik M, Bateman CC, Kolarik M, Stelinski LL, Jordal BH, Hulcr J (2015) The ambrosia symbiosis is specific in some species and promiscuous in others: evidence from community pyrosequencing. ISME J 9:126–138. https://doi.org/10.1038/ismej.2014.115

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol and Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054

Li Y, Huang Y-T, Kasson MT, Macias AM, Skelton J, Carlson PS, Yin M, Hulcr J (2018) Specific and promiscuous ophiostomatalean fungi associated with *Platypodinae* ambrosia beetles in the southeastern United States. Fungal Ecol 35:42–50. https://doi.org/10.1016/j.funeco.2018.06.006

Liebhold AM, MacDonald WL, Bergdahl D, Mastro VC (1995) Invasion by exotic forest pests: a threat to forest ecosystems. Forest Sci 41:a0001-z0001. https://doi.org/10.1093/forestscience/41.s1.a0001

López-Romero E, Reyes-Montes MdR, Pérez-Torres A, Ruiz-Baca E, Villagómez-Castro JC, Mora-Montes HM, Flores-Carreón A, Toriello C (2011) *Sporothrix schenckii* complex and sporotrichosis, an emerging health problem. Futur Microbiol 6:85–102. https://doi.org/10.2217/fmb.10.157

Lynn KMT, Wingfield MJ, Durán A, Marincowitz S, Oliveira LSS, De Beer ZW, Barnes I (2020) *Euwallacea perbrevis* (Coleoptera: Curculionidae: Scolytinae), a confirmed pest on *Acacia crassicarpa*in Riau, Indonesia, and a new fungal symbiont; *Fusarium rekanum* sp. nov. Antonie Van Leeuwenhoek 6:803–823. https://doi.org/10.1007/s10482-020-01392-8

Machingambi NM, Roux J, Dreyer LL, Roets F (2014) Bark and ambrosia beetles (*Curculionidae*: *Scolytinae*), their phoretic mites (Acari) and associated *Geosmithia* species (*Ascomycota*: *Hypocreales*) from *Virgilia* trees in South Africa. Fungal Biol 118:472–483. https://doi.org/10.1016/j.funbio.2014.03.006

Malacrino A, Rassati D, Schena L, Mehzabin R, Battisti A, Palmeri V (2017) Fungal communities associated with bark and ambrosia beetles trapped at international harbours. Fungal Ecol 28:44–52. https://doi.org/10.1016/j.funeco.2017.04.007

Massoumi-Alamouti S, Tsui CK, Breuil C (2009) Multigene phylogeny of filamentous ambrosia fungi associated with ambrosia and bark beetles. Mycol Res 113:822–835. https://doi.org/10.1016/j.mycres.2009.03.003

O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are non-orthologous. Mol Phylogenet Evol 7:103–116. https://doi.org/10.1006/mpev.1996.0376

Osorio JA, De Beer ZW, Wingfield MJ, Roux J (2016) Ophiostomatoid fungi associated with mangroves in South Africa, including *Ophiostoma palustre* sp. nov. Antonie Van Leeuwenhoek 109:1555–1571. https://doi.org/10.1007/s10482-016-0757-7

Ploetz RC, Hulcr J, Wingfield MJ, De Beer ZW (2013) Destructive tree diseases associated with ambrosia and bark beetles: black swan events in tree pathology? Plant Dis 97:856–872. https://doi.org/10.1094/PDIS-01-13-0056-FE

Rabaglia RJ, Dole SA, Cognato AI (2006) Review of American Xyleborina (*Coleoptera*: *Curculionidae*: *Scolytinae*) occurring north of Mexico, with an illustrated key. Ann Entomol Soc Am 99:1034–1056. https://doi.org/10.1603/0013-8746

Ratzeburg JTC (1837) Die Forst-insekten oder Abbildung und Beschreibung der in den Wäldern Preussens und den Nachbarstaaten als schädlich oder nützlich bekannt gewordenen Insekten. Nicola, Berlin

Rayner RW (1970) A mycological colour chart. Commonwealth Mycological Institute, Kew, Surrey and The British Mycological Society

Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 17:1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Saucedo J, Ploetz R, Carrillo D, Konkol J, Smith J, Rollins J, Ochoa S (2016) *Raffaelea arxii* may be the primary symbiont of *Xyleborus affinis* [abstract]. Conference Paper: American Phytopathological Society: Vol. 106, pp. 124-124

Saucedo J, Ploetz R, Konkol J, Ángel M, Mantilla J, Menocal O, Carrillo D (2018) Nutritional symbionts of a putative vector, *Xyleborus bispinatus*, of the laurel wilt pathogen of avocado, *Raffaelea lauricola*. Symbiosis 75:29–38. https://doi.org/10.1007/s13199-017-0514-3

Saucedo-Carabez J, Ploetz RC, Konkol J, Carrillo D, Gazis R (2018) Partnerships between ambrosia beetles and fungi: lineage-specific promiscuity among vectors of the laurel wilt pathogen, *Raffaelea lauricola*. Microb Ecol 76:925–940. https://doi.org/10.1007/s00248-018-1188-y

Schedl KE (1975) South African bark and timber beetles, 3. Contribution to the morphology and taxonomy of the Scolytoidea. Ann Transvaal Mus 29:275–281

Schedl KE (1982) *Scolytoidea* (*Coleoptera*), mainly from South Africa. Ann Transvaal Mus 33:277–286

Scott D, Du Toit J (1970) Three new *Raffaelea* species. Trans Br Mycol Soc 55:181–186. https://doi.org/10.1016/S0007-1536(70)80002-X

Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. Org Divers Evol 12:335–337. https://doi.org/10.1007/s13127-011-0056-0

Simmons DR, De Beer ZW, Huang Y-T, Bateman C, Campbell AS, Dreaden TJ, Li Y, Ploetz RC, Black A, Li H-F (2016) New *Raffaelea* species (ophiostomatales) from the USA and Taiwan associated with ambrosia beetles and plant hosts. IMA Fungus 7:265. https://doi.org/10.5598/imafungus.2016.07.02.06

Six DL (2012) Ecological and evolutionary determinants of bark beetle—fungus symbioses. Insects 3:339–366. https://doi.org/10.3390/insects3010339

Talbot PHB (1977) The *Sirex-Amylostereum-Pinus* Association. Annu Rev Phytopathol 15:41–54. https://doi.org/10.1146/annurev.py.15.090177.000353

Van der Linde JA, Six DL, De Beer WZ, Wingfield MJ, Roux J (2016) Novel ophiostomatalean fungi from galleries of *Cyrtogenius africus* (Scolytinae) infesting dying *Euphorbia ingens*. Antonie Van Leeuwenhoek 109:589–601. https://doi.org/10.1007/s10482-016-0661-1

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172:4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

White TJ, Bruns T, Lee S, Taylor J, Innis MA, Gelfand DH, Sninsky JJ, White TJ (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Academic Press, New York

Wood SL, Bright DE (1992) A catalog of scolytidae and *Platypodidae* (*Coleoptera*), part 2: taxonomic index. Great Basin Nat Mem 13:835–1557

Zipfel RD, De Beer ZW, Jacobs K, Wingfield BD, Wingfield MJ (2006) Multi-gene phylogenies define *Ceratocystiopsis* and *Grosmannia* distinct from *Ophiostoma*. Stud Mycol 55:75–97. https://doi.org/10.3114/sim.55.1.75

CHAPTER 3

The granulate ambrosia beetle, *Xylosandrus crassiusculus* (Coleoptera: Curculionidae, Scolytinae), and its fungal symbiont found in South Africa

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The granulate ambrosia beetle, *Xylosandrus crassiusculus* (Coleoptera: Curculionidae, Scolytinae), and its fungal symbiont found in South Africa

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Abstract

Xylosandrus crassiusculus (Motchulsky) is a native Asian ambrosia beetle that has been accidentally introduced to many countries of the world, presumably through the international movement of nursery, timber, and wood products. The species is known in various tropical African countries but only as far south as Tanzania on the African continent. In this study, we report *X. crassiusculus* and its fungal symbiont for the first time from South Africa. The species was identified using both morphological characters and COI sequence data. *Xylosandrus crassiusculus* were obtained from three different provinces of South Africa and represent two distinct haplotypes. The fungal symbiont, *Ambrosiella roeperi,* was isolated and identified using DNA sequencing and morphological characterization.

Key words: *Ambrosiella roeperi*, Ceratocystidaceae, invasive species, Xyleborini

Introduction

International trade in plant products has become a significant threat to sustainable agriculture and forestry because it facilitates the movement of exotic pest species (Colunga-Garcia *et al.* 2009; Wingfield *et al.* 2015; Fahrner & Aukema 2018). Bark and ambrosia beetles (Curculionidae: Scolytinae) are amongst the most frequently intercepted pest species at ports-of-entry, where they are found in various wood products including shipping and packaging material, logs and lumber (Haack 2001, 2006; Brockerhoff *et al.* 2006; Meurisse *et al.* 2019). These beetles represent some of the most threatening pest species affecting native and plantation trees globally and their accidental dissemination is of great concern. Yet little research has been conducted on bark and ambrosia beetles in South Africa, subsequent to the last work of Karl Schedl in 1980 (Wood & Bright 1992).

The granulate ambrosia beetle, *Xylosandrus crassiusculus* (Motchulsky) is one of the most globally widespread scolytine species (Landi *et al.* 2017). It is believed to be of Asian origin and has been introduced into many parts of Europe and the Americas (Pennachio *et al.* 2003; Fletchman *et al.* 2016; Gallego *et al.* 2017; Landi *et al.* 2017). Its highly polyphagous nature implies that it is a potential threat to both natural forest ecosystems and plantation forestry, although it is currently not considered a major pest in its native range (Pennachio *et al.* 2003; Gallego *et al.* 2017; EPPO 2020).

Xylosandrus crassiusculus, like other ambrosia beetles, lives in association with a fungal symbiont. This ascomycete fungus, *Ambrosiella roeperi*, serves as a source of nutrition to larvae and developing adult beetles (Harrington *et al.* 2014). The fungus resides in the Ceratocystidaceae (de Beer *et al.* 2014, Harrington *et al.* 2014) that accommodates tree pathogens and common agents of sap stain, frequently transmitted by scolytine and nitidulid (Coleoptera: Cucujoidea) beetles (Juzwik & French 1983; Heath *et al.* 2009; de Beer *et al.* 2014; Mayers *et al.* 2015, 2020; Wingfield *et al.* 2017).

The recent discovery of the destructive polyphagous shot hole borer (PSHB), *Euwallacea fornicatus* (Eichhoff), in South Africa has led to a renewed interest in bark and ambrosia beetles (Paap *et al.* 2018). Concern regarding

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the PSHB has resulted in many samples of scolytine-infested wood being examined, including avocado (*Persea americana*) and macadamia (*Macadamia integrifolia x M. tetraphylla*) logs, but much of the material was infested with species other than PSHB. Here we present the first record of *X. crassiusculus* in South Africa with information on its fungal symbiont.

Materials and methods

Beetle specimens

Insect-infested wood samples submitted by members of the public were placed in emergence chambers and the emerging beetles were collected. From this material, an unidentified species of Xyleborini was collected that was also found during field collections carried out at two locations in Tzaneen, Limpopo Province (23° 42' 29.491" S, 30° 5' 57.638" E & 23° 44' 29.491" S, 30° 11' 15.417" E) using a Bambara beetle trap (Hulcr & McCoy 2015) during the summer of 2018/2019. All beetle specimens were maintained in 96% ethanol in the Scolytinae collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa until study.

Fungal isolations

Fungal isolates were obtained from two living beetles collected from one of the sites in Tzaneen, Limpopo Province (23° 42' 29.491" S, 30 5' 57.638" E) using a modified Bambara beetle trap. The trap contained a wire mesh insert (gap size of 0.5 mm) between the entrance and collection zone, preventing the insects from coming into contact with the ethanol lure. The beetles were allowed to walk over the surface of Malt Extract Agar plates (MEA: 2% Biolab malt extract and 2% Difco agar) amended with streptomycin (400 mg/L, Sigma-Aldrich, Missouri, United States) in 65 mm Petri dishes. The plates were incubated at ambient temperature and frequently inspected for fungal growth.

Once fungal growth was observed, isolates were purified by transferring hyphal tips to new MEA plates. Three isolates with culture morphologies resembling an *Ambrosiella* sp. were obtained and used for further morphological and DNA sequence-based characterization. Representative pure cultures of the fungus are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (Table 1).

TABLE 1. Fungal isolatesvobtained in this study and GenBank accession numbers of ITS sequences used in phylogenetic analysis

Species	Collection number	Country	Accession
Ambrosiella roeperi	WN2019-09-94	South Africa	MT230429
	CMW 55799	South Africa	MT230430
	CMW 55800	South Africa	MT230431
	B242U4	Taiwan	LC175300
	B241U7	Taiwan	LC175299
	B239U1	Taiwan	LC175297
	CBS135864	USA	NR_154684
	JH12076	USA	MK118927
Ambrosiella catenulata	M257	Taiwan	KR611325
Ambrosiella grosmanniae	CBS137359	USA	NR_154702
Ambrosiella batrae	CBS139735	USA	NR_154700
Ambrosiella hartigii	C1573	Germany	KF669873
Ambrosiella xylebori	CBS110.61	Cote d'Ivoire	NR_144921
Ambrosiella remansi	M290	Madagascar	KX342068
Ambrosiella nakashimae	CBS139739	USA	NR_154701
Ambrosiella beaveri	CBS121750	USA	NR_154685
Meredithiella norrisii	CBS139737	USA	NR_154183
Catunica adiposa	CBS138.34	Netherlands	MH855471
	CBS136.34	Japan	MH855470

Morphological observations

Beetle specimens were examined using an automated Zeiss Discovery V12 stereomicroscope (Zeiss, Oberkocken, Germany) with an affixed Zeiss Axiocam IcC 5 camera. The ventral, lateral and dorsal aspects of specimens were examined up to 100X magnification. Focus-stacked photographs were produced using Helicon Focus v. 5 (Helicon-Soft, Kharkiv, Ukraine) with up to 30 different images. Specimens were identified to genus level using the taxonomic key from Faccoli (2008) and to species using the key from Dole & Cognato (2010).

Fungal isolates were examined using a Nikon Eclipse Ni compound microscope with an affixed Nikon Ds-Ri2 camera (Nikon, Tokyo, Japan). Fungal structures were removed from actively growing culture plates and mounted in 80% lactic acid solution and examined using bright field and differential interference contrast microscopy. Photographs and measurements were taken using the software Nikon RIS Elements.

DNA sequence-based identification of beetle specimens

Based on initial morphological examination, representative beetle specimens from different localities and hosts were selected for DNA sequencing and molecular characterization. DNA was extracted using the Macherey-Nagel NucleoSpin Tissue kit (Macherey-Nagel, Dueren, Germany) from the heads of individual beetles, which had been removed using a sterilized scalpel. One of the beetles from which a fungal isolate had been obtained was treated in a similar manner to confirm its morphological identification. Extraction of DNA was carried out following the manufacturer's suggested protocols, except for the final elution volume that was reduced to 60 µl. PCR amplification of the mitochondrial cytochrome oxidase 1 (COI) region was done for all specimens using the primers LCO1490 and HCO2198 (Folmer *et al.* 1994).

Due to the limited sequence availability for bark and ambrosia beetles currently available in public repositories, two specimens were selected for sequencing of additional gene regions. For these specimens, PCR amplification of the nuclear ribosomal 28S and CAD regions was carried out using the primer pairs 3665 and 4068 (Belshaw & Quicke 1997; Cognato 2013) and apCADforB2 and apCADrevlmod (Danforth *et al.* 2006), respectively. All PCR reactions were carried out as described by Cognato (2013) in 25 µL reaction volumes.

PCR products were cleaned using ExoSAP-IT PCR Product Clean-up Reagent (ThermoFisher Scientific, Massachusetts, United States). Sequencing reactions were carried out for both the forward and reverse primers using the BigDye Terminator 3.1 Cycle Sequencing protocol (Applied Biosystems, California, United States) at an annealing temperature of 55°C. Products were purified using the sodium acetate and ethanol precipitation method and submitted to the DNA sanger sequencing facility based at the University of Pretoria for analyses on an ABI PRISM3500 Genetic Analyzer (Applied Biosystems, California, United States). Sequence Scanner v 1.0 (https://sequence-scanner-software.software.informer.com/) was used to assess the quality of the sequence data and for editing. Sequences were deposited in NCBI GenBank (www.ncbi.nlm.nih.gov/Genbank) and the accession numbers are provided in Table 2.

The consensus COI sequences were used in BLASTN searches against the non-redundant nucleotide sequence database available in NCBI GenBank for putative identification of species. Based on these identifications and data availability, a dataset was prepared using reference sequences from Landi *et al.* (2017) (Table 3). The dataset was prepared using MEGA v. 6.06 (Tamura *et al.* 2013) and alignments were done using the online version of MAFFT v. 7 (Katoh & Standley 2013). Maximum likelihood analyses were performed using the software raxmlGUI v. 2 (Silvestro & Michalak 2012; Elder *et al.* 2019) with the General Time Reversible + GAMMA (GTR+G) nucleotide substitution model and 1000 bootstrap replicates.

TABLE 2. *Xylosandrus crassiusculus* specimens used in phylogenetic analysis

Specimen number	Country	Locality	Host	Accession		
				COI	28S	CAD
F131	South Africa	Northern Cape	Avocado	MT230103		
F002	South Africa	Limpopo	Avocado	MT230099		
F178	South Africa	Kwazulu-Natal	Macadamia	MT230102	MT229960	MT239349
F102	South Africa	Unknown	Macadamia	MT230100		
XC3	South Africa	Limpopo	Ethanol trap	MT230104		
WNB0060	South Africa	Limpopo	Ethanol trap	MT230101	MT229959	MT239348

TABLE 3. Reference sequences used in phylogenetic analysis

Species	Specimen number	Country	Locality	Accession
Xylosandrus crassiusculus	Xyocra07	USA	Maryland	GU808710
	Xyocra08	USA	North Carolina	GU808711
	Xyocra01	Madagascar	Montagne d'Akirindro	GU808708
	Xyocra02	Thailand	Chiangmai	GU808709
	(SYC)2079-14	French Polynesia	Tahiti	SYC2079-14*
	(BMNH)1043087	Panama		GBMNA9640-19*
	R2AC91	Malaysia	Selangor	MBIO132-13*
	(BIOUG23741-)H07	Bangladesh	Chittagong	GMBCG253-15*
	(BIOUG28065-)A02	Costa Rica	Guanacaste	GMABV258-16*
	LL6	Argentina		GBMIN51869-17*
	(BC-PNEF-PSFOR)0863	France	Cote d'Azur	PSFOR1233-17*
	(SC)08506	French Polynesia	Tahiti	KX055197
	(SC)03268	French Polynesia	Moorea	KX055198
	Sax310	Japan	Okinawa	MN620077
	Sax180	Vietnam	Dong Nai	MN620073
	Sax351	Vietnam	Ninh Bing	MN620078
	Sax29	Vietnam	Cao Bang	MN620070
	Sax245	China	Hong Kong	MN620075
	Sax179	Vietnam	Thua Thien-Hue	MN620072
	Sax230	Taiwan		MN620074
	Sax104	India	Dehra Dun	MN620071
	Sax281	China	Shanghai	MN620076
	(BIOUG26285-)B04	Canada	Ontario	MF637141
	(BIOUG:10BBCOL-)0245	USA		HQ983950
	(BIOUG:10BBCOL-)0244	USA		HQ983949
	(BIOUG:10BBCOL-)0243	USA		HQ983948
Cnestus ater	Xyoate	Borneo	Danum Valley	GU808705
C. improcerus	Xyoimp	Borneo	Danum Valley	GU808715
X. morigerus	Xyomor01	Papua New Guinea		GU808717

*Sequences were obtained from the BOLD database
()Parts of specimen names omitted from phylogenetic tree

DNA sequence-based identification of fungal isolates

DNA was extracted from freeze dried mycelium of the three fungal isolates obtained using the extraction method described in de Beer *et al.* (2014). PCR amplification was carried out for the internal transcribed spacer region (ITS) using the primer pair ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). PCR reactions were carried out as described by Duong *et al.* (2012) in 25 µL reaction volumes and an annealing temperature of 55°C. PCR products were cleaned using ExoSAP-IT PCR Product Clean-up Reagent and sequencing PCR reactions, precipitation and quality assessment was carried out as described above for the beetle specimens. The generated sequences were deposited in NCBI GenBank with the accession numbers provided in Table 1.

The ITS consensus sequences were used in BLASTN searches against the non-redundant nucleotide sequence database available in NCBI GenBank for putative identification of species. Based on these identifications, a dataset (Table 1) was prepared using reference sequences from Mayers *et al.* (2020). This dataset was prepared and analysed using the same method described for the beetle specimens.

Results

Identification of beetles

The COI sequences generated for the beetles led to their tentative identification as a species of *Xylosandrus* Reitter and were very similar to those of *X. crassiusculus* available in NCBI GenBank. Maximum likelihood phylogenetic analyses (Fig. 1A) grouped the specimens from Kwazulu-Natal and the Northern Cape of South Africa with a diverse group of specimens from geographically distant locations including Panama, Thailand, and Madagascar. The remaining specimens from Limpopo grouped with specimens from Argentina, France, and the United States.

The morphological characteristics of the beetle specimens (Fig. 1B) matched those of *X. crassiusculus* (Dole & Cognato 2010). The COI sequence generated for beetle specimen XC3 from which two of the three fungal isolates were obtained, matched those of the sequences for other *X. crassiusculus* specimens from Limpopo.

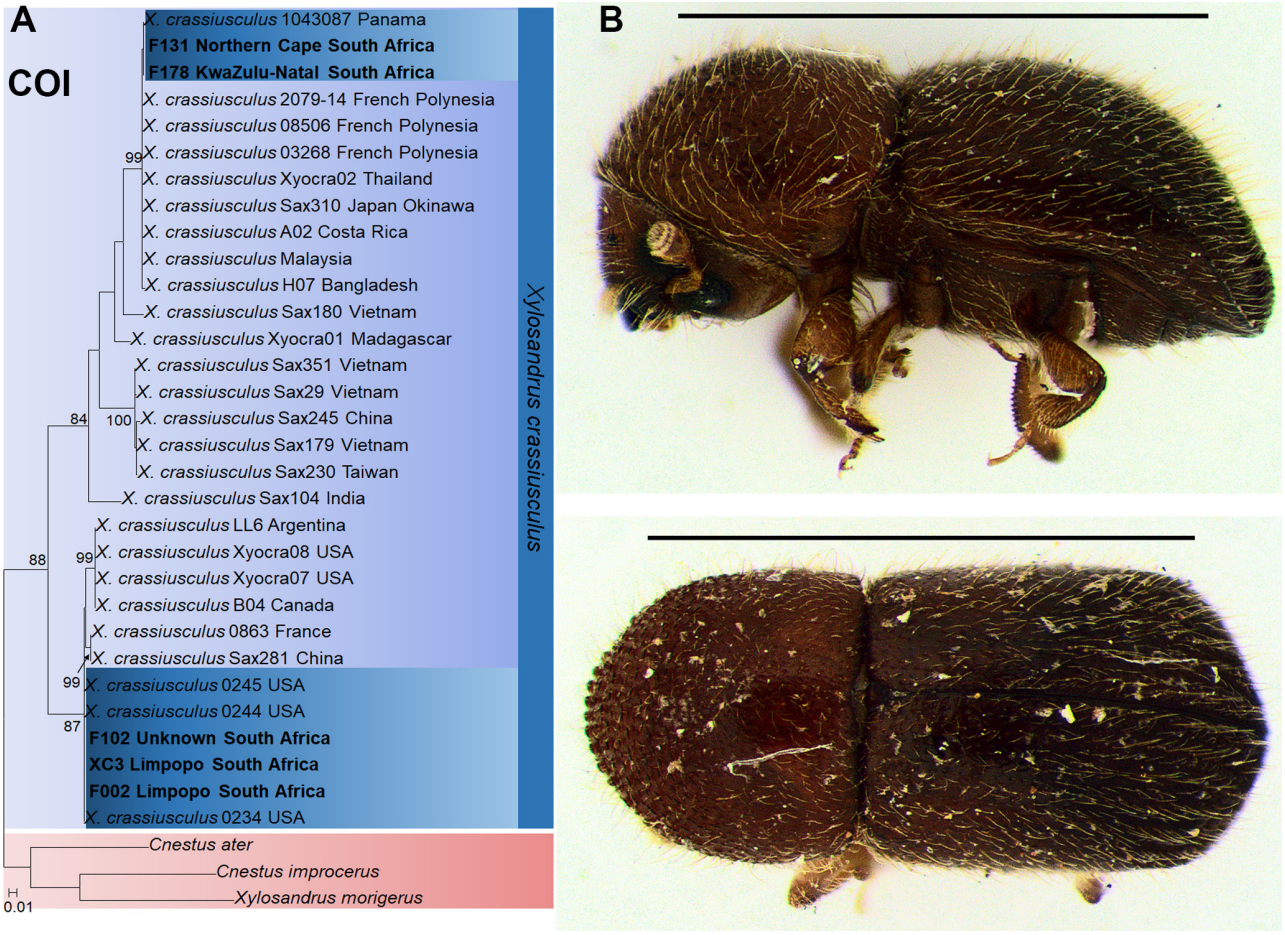

FIGURE 1. Phylogenetic and morphological identification of *Xylosandrus crassiusculus*. **A** Phylogram produced from maximum likelihood analysis using RAxML for the COI region of specimens of *X. crassiusculus*. Specimens sequenced in this study are highlighted in bold. Nodes with bootstrap support higher than 75 are indicated. **B** Lateral and dorsal view of a *X. crassiusculus* female. Scale bars: 2 mm.

Identification of fungal isolates

Three isolates resembling a species of *Ambrosiella* were obtained from two living specimens of *X. crassiusculus*. The generated ITS sequences for these isolates were identical to several isolates of *A. roeperi* from Taiwan and differed from the ex-holotype isolate from the USA by only one base-pair. Maximum likelihood phylogenetic analysis (Fig. 2A) including five isolates of *A. roeperi* and other representative *Ambrosiella* spp., as well as three outgroup Ceratocystidaceae species, grouped the isolates from South Africa together with other *A. roeperi* isolates. All isolates in this study representing *A. roeperi* grouped closely in a distinct clade separated from other *Ambrosiella* species. Morphological characteristics of the isolates (Fig. 2B) matched those known for *A. roeperi* (Harrington *et al.* 2014).

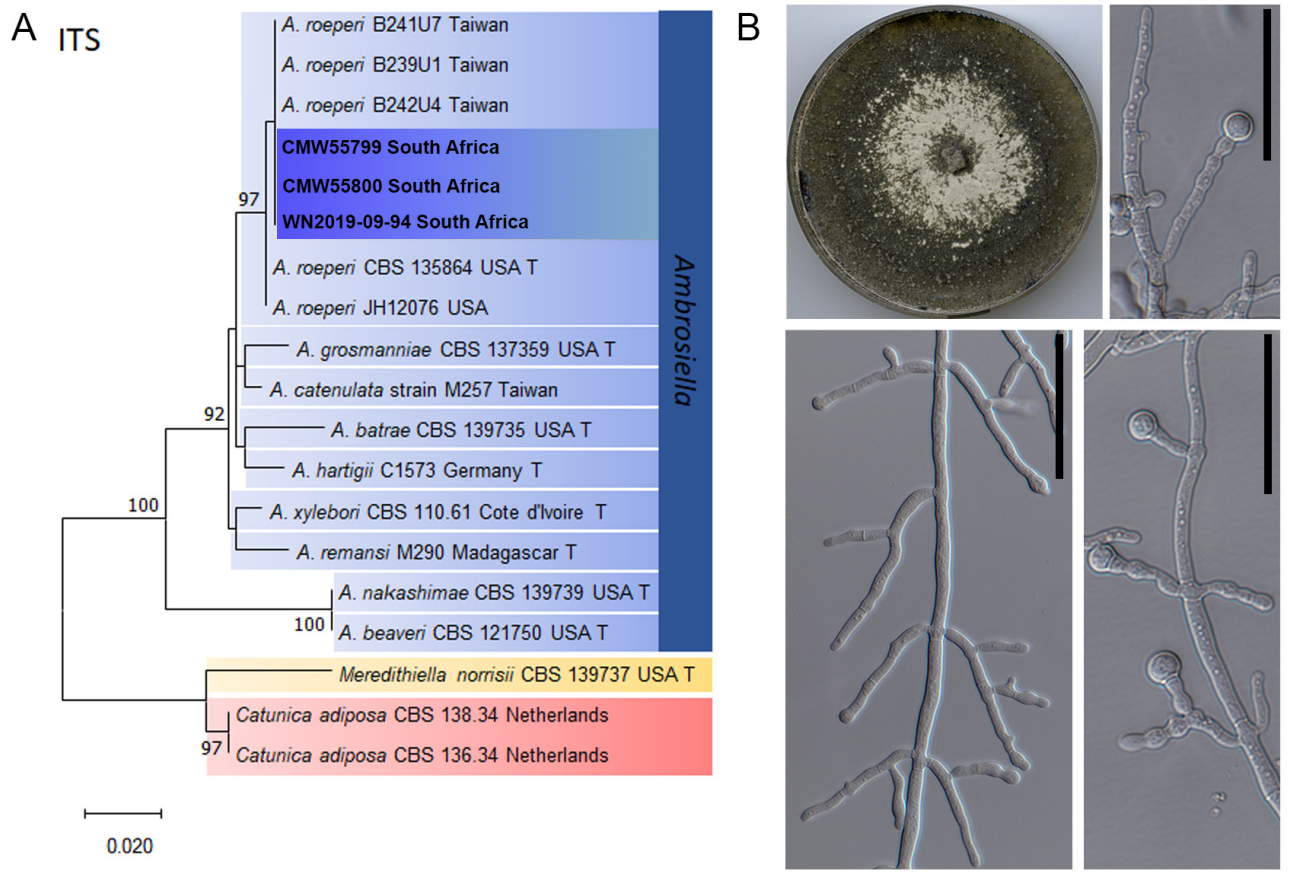

FIGURE 2. Phylogenetic and morphological identification of *Ambrosiella roeperi.* **A** Phylogram produced from maximum likelihood analysis using RAxML for the ITS region of isolates of *A. roeperi.* Isolates obtained and sequenced in this study are highlighted in bold. Nodes with bootstrap support higher than 75 are indicated. **B** Culture morphology of a two-week-old isolate grown on MEA (top left) and microscopic characters of *A. roeperi*. Scale bars: 50µm.

Discussion

Detection of the polyphagous shot hole borer (Paap *et al.* 2018) in South Africa has heightened research interest in bark and ambrosia beetles and resulted in many scolytine-infested wood samples being collected and submitted for identification. This study, employing both morphological characters and DNA sequence data led to the identification of *X. crassiusculus,* which is here recorded in South Africa for the first time. Likewise, we were able to isolate and identify the fungal symbiont of the collected *X. crassiusculus* specimens as *A. roeperi*.

Maximum likelihood phylogenetic analysis of the COI region suggested that two haplotypes of *X. crassiusculus* are present in South Africa. Sequence data for the ribosomal 28S and CAD regions also supported this conclusion. However, the paucity in DNA sequence data (Landi *et al.* 2018) and high variation seen in the COI gene of xyleborine ambrosia beetles (Cognato *et al.* 2020) precluded us from determining whether these haplotypes arrived in South Africa due to two independent introduction events or whether they could have spread south from an unknown introduction location elsewhere on the African continent.

Xylosandrus crassiusculus and its fungal symbiont could pose a threat to some tree species in its introduced range including South Africa. This has for example been seen for the carob tree (*Ceratonia siliqua*) in parts of Europe (Gallego *et al.* 2017). It is also relevant to recognise that, once established in a region, ambrosia beetles such as *X. crassiusculus* are difficult to control (Ranger *et al.* 2016). This relates not only to their ability to spread rapidly but also due, in some cases such as *X. crassiusculus*, to them being haplo-diploid, enabling a single introduced female to start an invasion (Jordal *et al.* 2000), and their polyphagous nature, allowing them to survive in a large variety different hosts (Kirkendall & Faccoli 2010). These facts are particularly relevant in South Africa, where *X. crassiusculus* is already widespread and has now been found infesting avocado and macadamia trees, both impor-

tant to local agriculture. However, it must be noted that both tree species were stressed at the time of infestation due to environmental factors, so the effect that *X. crassiusculus* might have on these industries remains unclear.

Conclusions

The south Asian ambrosia beetle *X. crassiusculus* is a successful invasive species that has been accidentally introduced into many tropical and subtropical regions of the world. These include 15 countries of sub-Saharan Africa stretching from Mauritania in the north to the islands of Mauritius and Madagascar in the south (Wood & Bright 1992; Dole & Cognato 2010; EPPO 2020). *Xylosandrus crassiusculus* has been present on mainland Africa for many years but the present study represents the first record of both the insect and its fungal symbiont *A. roeperi* in South Africa. Its presence infesting important agronomic tree crops such as macadamia and avocado suggests that it requires further study.

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References

Belshaw, R. & Quicke, D.L.J. (1997) A molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae). *Molecular Phylogenetics and Evolution*, 7 (3), 281–293.
https://doi.org/10.1006/mpev.1996.0400

Brockerhoff, E.G., Bain, J., Kimberley, M. & Knížek, M. (2006) Interception frequency of exotic bark and ambrosia beetles (Coleoptera: Scolytinae) and relationship with establishment in New Zealand and worldwide. *Canadian Journal of Forest Research*, 36 (2), 289–298.
https://doi.org/10.1139/x05-250

Cognato, A.I. (2013) Molecular phylogeny and taxonomic review of Premnobiini Browne, 1962 (Coleoptera: Curculionidae: Scolytinae). *Frontiers in Ecology and Evolution*, 1 (1), 1–12.
https://doi.org/10.3389/fevo.2013.00001

Cognato, A.I., Sari, G., Smith, S.M., Beaver, R.A., Li, Y., Hulcr, J., Jordal, B.H., Kajimura, H., Lin, C-S., Pham, T.H., Singh, S. & Sittichaya, W. (2020) The essential role of taxonomic expertise in the creation of DNA databases for the identification and delimitation of Southeast Asian ambrosia beetle species (Curculionidae: Scolytinae: Xyleborini). *Frontiers in Ecology and Evolution*, 8 (27). [published online]
https://doi.org/10.3389/fevo.2020.00027

Colunga-Garcia, M., Haack, R.A. & Adelaja, A.O. (2009) Freight transportation and the potential for invasions of exotic insects in urban and periurban forests of the United States. *Journal of Economic Entomology*, 102 (1), 237–246.
https://doi.org/10.1603/029.102.0133

Danforth, B.N., Fang, J. & Sipes, S. (2006) Analysis of family–level relationships in bees (Hymenoptera: Apiformes) using 28S and two previously unexplored nuclear genes: CAD and RNA polymerase II. *Molecular Phylogenetics and Evolution*, 39 (2), 358–372.
https://doi.org/10.1016/j.ympev.2005.09.022

de Beer, Z.W., Duong, T.A., Barnes, I., Wingfield, B.D. & Wingfield, M.J. (2014) Redefining *Ceratocystis* and allied genera. *Studies in Mycology*, 79, 187–219.
https://doi.org/10.1016/j.simyco.2014.10.001

Dole, S.A. & Cognato, A.I. (2010) Phylogenetic revision of *Xylosandrus* Reitter (Coleoptera: Curculionidae: Scolytinae: Xyleborina). *Proceedings of the California Academy of Sciences*, 61 (7), 451–545.

Duong, T.A., de Beer, Z.W., Wingfield, B.D. & Wingfield, M.J. (2012) Phylogeny and taxonomy of species in the *Grosmannia serpens* complex. *Mycologia*, 104 (3), 715–732.
https://doi.org/10.3852/11-109

Edler, D., Klein, J., Antonelli, A. & Silvestro, D. (2019) raxmlGUI 2.0 beta: a graphical interface and toolkit for phylogenetic analyses using RAxML. *bioRxiv*, 800912.

https://doi.org/10.1101/800912
European and Mediterranean Plant Protection Organization (EPPO) (2020) EPPO alert list—*Xylosandrus crassiusculus* (Coleoptera: Scolytinae). Available from: https://www.eppo.int/ACTIVITIES/plant_quarantine/alert_list_insects/xylosandrus_crassiusculus (accessed 7 July 2020)
Faccoli, M. (2008) First record of *Xyleborus atratus* Eichhoff from Europe, with an illustrated key to the European Xyleborini (Coleoptera: Curculionidae: Scolytinae). *Zootaxa*, 1772 (1), 55–62.
https://doi.org/10.11646/zootaxa.1772.1.2
Fahrner, S. & Aukema, B.H. (2018) Correlates of spread rates for introduced insects. *Global Ecology and Biogeography*, 27 (6), 734–743.
https://doi.org/10.1111/geb.12737
Flechtmann, C.A. & Atkinson, T.H. (2016) First records of *Xylosandrus crassiusculus* (Motschulsky) (Coleoptera: Curculionidae: Scolytinae) from South America, with notes on its distribution and spread in the New World. *The Coleopterists Bulletin*, 70 (1), 79–83.
https://doi.org/10.1649/072.070.0109
Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3 (5), 294–299.
Gallego, D., Lencina, J.L., Mas, H., Cever, J. & Faccoli, M. (2017) First record of the granulate ambrosia beetle, *Xylosandrus crassiusculus* (Coleoptera: Curculionidae, Scolytinae), in the Iberian Peninsula. *Zootaxa*, 4273 (3), 431–434.
https://doi.org/10.11646/zootaxa.4273.3.7
Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. *Molecular Ecology*, 2, 113–118.
https://doi.org/10.1111/j.1365-294X.1993.tb00005.x
Haack, R.A. (2001) Intercepted Scolytidae (Coleoptera) at US ports of entry: 1985–2000. *Integrated Pest Management Reviews*, 6 (3), 253–282.
https://doi.org/10.1023/A:1025715200538
Haack, R.A. (2006) Exotic bark- and wood-boring Coleoptera in the United States: recent establishments and interceptions. *Canadian Journal of Forest Research*, 36 (2), 269–288.
https://doi.org/10.1139/x05-249
Harrington, T.C., McNew, D., Mayers, C.G., Fraedrich, S.W. & Reed, S.E. (2014) *Ambrosiella roeperi* sp. nov. is the mycangial symbiont of the granulate ambrosia beetle, *Xylosandrus crassiusculus*. *Mycologia*, 106 (4), 835–845.
https://doi.org/10.3852/13-354
Heath, R.N., Wingfield, M.J., Van Wyk, M. & Roux, J. (2009) Insect associates of *Ceratocystis albifundus* and patterns of association in a native savanna ecosystem in South Africa. *Environmental Entomology*, 38 (2), 356–364.
https://doi.org/10.1603/022.038.0207
Hulcr, J. & McCoy, N. (2015) Catching beetles. Available from: http://www.ambrosiasymbiosis.org/ambrosia-beetles/catching-beetles/ (accessed 10 June 2020)
Jordal, B.H., Normark, B.B. & Farrell, B.D. (2000) Evolutionary radiation of an inbreeding haplodiploid beetle lineage (Curculionidae, Scolytinae). *Biological Journal of the Linnean Society*, 71 (3), 483–499.
https://doi.org/10.1111/j.1095-8312.2000.tb01270.x
Jordal, B.H., Gillespie, J.J. & Cognato, A.I. (2008) Secondary structure alignment and direct optimization of 28S rDNA sequences provide limited phylogenetic resolution in bark and ambrosia beetles (Curculionidae: Scolytinae). *Zoologica Scripta*, 37 (1), 43–56.
https://doi.org/10.1111/j.1463-6409.2007.00306.x
Juzwik, J. & French, D.W. (1983) *Ceratocystis fagacearum* and *C. piceae* on the surfaces of free–flying and fungus–mat–inhabiting nitidulids. *Phytopathology*, 73 (8), 1164–1168.
https://doi.org/10.1094/Phyto-73-1164
Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, 30, 772–780.
https://doi.org/10.1093/molbev/mst010
Kirkendall, L.R. & Faccoli, M. (2010) Bark beetles and pinhole borers (Curculionidae, Scolytinae, Platypodinae) alien to Europe. *ZooKeys*, 56, 227–251.
https://doi.org/10.3897/zookeys.56.529
Landi, L., Gómez, D., Braccini, C.L., Pereyra, V.A., Smith, S.M. & Marvaldi, A.E. (2017) Morphological and molecular identification of the invasive *Xylosandrus crassiusculus* (Coleoptera: Curculionidae: Scolytinae) and its South American range extending into Argentina and Uruguay. *Annals of the Entomological Society of America*, 110 (3), 344–349.
https://doi.org/10.1093/aesa/sax032
Mayers, C.G., McNew, D.L., Harrington, T.C., Roeper, R.A., Fraedrich, S.W., Biedermann, P.H., Castrillo, L.A. & Reed, S.E. (2015) Three genera in the Ceratocystidaceae are the respective symbionts of three independent lineages of ambrosia beetles with large, complex mycangia. *Fungal Biology*, 119 (11), 1075–1092.
https://doi.org/10.1016/j.funbio.2015.08.002
Mayers, C.G., Harrington, T.C., Masuya, H., Jordal, B.H., McNew, D.L., Shih, H-H., Roets, F. & Kietzka, G.J. (2020) Patterns

of coevolution between ambrosia beetle mycangia and the Ceratocystidaceae, with five new fungal genera and seven new species. *Persoonia–Molecular Phylogeny and Evolution of Fungi*, 44, 41–66.
https://doi.org/10.3767/persoonia.2020.44.02

Meurisse, N., Rassati, D., Hurley, B.P., Brockerhoff, E.G. & Haack, R.A. (2019) Common pathways by which non-native forest insects move internationally and domestically. *Journal of Pest Science*, 92 (1), 13–27.
https://doi.org/10.1007/s10340-018-0990-0

Paap, T., de Beer, Z.W., Migliorini, D., Nel, W.J. & Wingfield, M.J. (2018) The polyphagous shot hole borer (PSHB) and its fungal symbiont *Fusarium euwallaceae*: a new invasion in South Africa. *Australasian Plant Pathology*, 47 (2), 231–237.
https://doi.org/10.1007/s13313-018-0545-0

Pennacchio, F., Roversi, P., Francardi, V. & Gatti, E. (2003) *Xylosandrus crassiusculus* (Motschulsky) a bark beetle new to Europe (Coleoptera Scolytidae). *Redia*, 86 (2), 77–80.

Ranger, C.M., Reding, M.E., Schultz, P.B., Oliver, J.B., Frank, S.D., Addesso, K.M., Hong Chong, J., Sampson, B., Werle, C., Gill, S. & Krause, C. (2016) Biology, ecology, and management of non-native ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) in ornamental plant nurseries. *Journal of Integrated Pest Management*, 7 (1), 1–23.
https://doi.org/10.1093/jipm/pmw005

Silvestro, D. & Michalak, I. (2012) raxmlGUI: a graphical front–end for RAxML. *Organism Diversity & Evolution*, 12, 335–337.
https://doi.org/10.1007/s13127-011-0056-0

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30 (12), 2725–2729.
https://doi.org/10.1093/molbev/mst197

White, T.J., Bruns, T., Lee, S., Taylor, J., Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In: PCR protocols: a guide to methods and applications*, Academic Press, New York, pp. 315–322.
https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wingfield, M.J., Brockerhoff, E., Wingfield, B.D. & Slippers, B. (2015) Planted forest health: the need for a global strategy. *Science*, 349 (6250), 832–836.
https://doi.org/10.1126/science.aac6674

Wingfield, M.J., Barnes, I., de Beer, Z.W., Roux, J., Wingfield, B.D. & Taerum, S.J. (2017) Novel associations between ophiostomatoid fungi, insects, and tree hosts: current status—future prospects. *Biological Invasions*, 18 (4), 3215–3228.
https://doi.org/10.1007/s10530-016-1084-7

Wood, S.L. & Bright, D.E. (1992) A Catalog of Scolytidae and Platypodidae (Coleoptera), Part 2: Taxonomic Index. *The Great Basin Naturalist Memoirs*, 13, 1–1553.

CHAPTER 4

Phylogenetic and phylogenomic analyses reveal two new genera and three new species of ophiostomatalean fungi from termite-fungus combs

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Phylogenetic and phylogenomic analyses reveal two new genera and three new species of ophiostomatalean fungi from termite-fungus combs

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1. Abstract

The Ophiostomatales (*Ascomycota*) accommodates more than 300 species characterized by similar morphological adaptations to arthropod dispersal. Most species in this order are wood-inhabiting fungi associated with bark or ambrosia beetles. However, a smaller group of species occur in other niches such as in soil and *Protea* infructescences. Recent surveys of *Termitomyces* fungus gardens (fungus combs) of fungus-growing termites led to the discovery of characteristic ophiostomatalean-like fruiting structures. In this study, these ophiostomatalean-like fungi were identified using morphological characteristics, conventional molecular markers and whole genome sequencing. In addition, the influence of the extracts derived from various parts of *Termitomyces* combs on the growth of these fungi in culture was considered. Based on phylogenomic analyses, two new genera (*Intubia* and *Chrysosphaeria*) were introduced to accommodate these Ophiostomatalean species. Phylogenetic analyses revealed that the isolates resided in three well-supported lineages, and these were described as three new species (*Intubia macrotermitinarum*, *I. oerlemansii* and *Chrysosphaeria jan-nelii*). Culture-based studies showed that these species do not depend on the *Termitomyces* comb material for growth.

2. Key Words

Fungus growing termites, Insect associated fungi, *Termitomyces*, Ophiostomataceae, 3 new species, 2 new genera

3. Introduction

The Ophiostomatales was first described in the late 1900's to accommodate the single family, Ophiostomataceae, which at the time included *Ceratocystis*, *Ceratocystiopsis*, *Ophiostoma*, and *Sphaeronaemella* (Benny & Kimbrough 1980). These genera share morphologically similar ascomata and ascospores that are adapted to arthropod dispersal (Malloch & Blackwell 1993). However, early DNA based phylogenetic studies showed that species of *Ophiostoma* and *Ceratocystis* are not closely related and reside in two different orders (Ophiostomatales and Microascales) of the Sordariomycetes (Hausner et al. 1993; De Beer et al. 2013a). Subsequently, numerous phylogenetic studies of the Ophiostomatales have been done; each including larger numbers of taxa and additional gene regions to address various questions surrounding the taxonomy and generic placement of many species (Spatafora & Blackwell 1994; Jacobs et al. 2001; Zipfel et al. 2006; Massoumi Alamouti et al. 2009; De Beer et al. 2016a).

De Beer & Wingfield (2013) provided an extensive review of the Ophiostomatales based on the "one fungus one name" principles (Hawksworth et al. 2011). Their phylogenetic analyses, including all published Ophiostomatales sequences in NCBI GenBank at the time, recognized six genera. However, the authors concluded that sequences for a greater number of gene regions and species were needed to resolve the generic boundaries of some lineages. Based on various revisions, the order now accommodates 12 genera including *Afroraffaelea*, *Aureovirgo*, *Ceratocystiopsis*, *Estyea*, *Fragosphaeria*, *Graphilbum*, *Grosmannia sensu latu* (s.l.), *Hawksworthiomyces*, *Leptographium* s.l., *Ophiostoma* s.l., *Sporothrix*, and *Raffaelea* (De Beer et al. 2016a; De Beer et al. 2016b; Bateman et al. 2017).

Most of the genera in the Ophiostomatales, especially *Ophiostoma* s.l., *Leptographium* s.l., *Ceratocystiopsis*, *Grosmannia* and *Graphilbum*, include species that are commonly associated with Scolytine bark beetles or their galleries (Six 2012; De Beer & Wingfield 2013; Kirisits 2013). However, in addition to these bark beetle associates, four other ecologically distinct groups have been identified. Two of these are species of *Raffaelea* and *Afroraffaelea* (De Beer et al. 2013a; Bateman et al. 2017), which are symbionts of ambrosia beetles where they are cultivated within the beetle galleries as a food

source (Harrington et al. 2010; Six 2012). Another group of species reside in *Sporothrix* (De Beer et al. 2016a), which are associated with mites, soil, *Protea* infructescences and a few species that are opportunistic human and animal pathogens (De Lima Barros et al. 2004; De Meyer et al; 2008; Roets et al. 2010; Hofstetter et al. 2013). A fourth ecologically distinct group accommodates species of *Hawksworthiomyces*, which appear to have the characteristic ability to break down the constituents that make up wood (De Meyer et al. 2008; De Beer et al. 2016b).

Ascocarps resembling ophiostomatalean fungi were observed on *Macrotermes natalensis Termitomyces* fungus combs in previous studies and fungal cultures were successfully isolated from this material. The aims of this study were (1) to identify these ophiostomatalean-like fungi based on conventional molecular markers, whole genome sequencing and morphological characteristics and (2) to consider if extracts of *Termitomyces* combs have an effect on their growth and development.

4. Materials and methods

4.1. Fungal isolations

Termitomyces combs were incubated in the absence of termites for up to two months, during which they were inspected multiple times for ophiostomatalean-like fruiting bodies. When observed, single spore drops were lifted from the tips of fruiting bodies using sterilized fine-tipped tweezers (Dumont®, Montignez, Switzerland) and transferred onto malt extract agar (MEA) plates containing streptomycin and cycloheximide (2 % malt extract and 2 % Difco® agar, Biolab, Midrand, South Africa, 0.04 % streptomycin and 0.05 % cycloheximide, Sigma-Aldrich, Kempton Park, South Africa). The plates were incubated for 7–10 d at ambient temperature (~20°C) until fungal colonies were visible. The colonies were then inspected and those with ophiostomatalean-like morphology were sub-cultured by transferring hyphal tips from the edge of an actively growing colony to a new MEA plate. The sub-culturing was repeated until pure cultures were obtained. Pure cultures were maintained on MEA, and representatives were submitted to the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa and the Westerdijk Fungal Biodiversity Institute (CBS-KNAW), Utrecht, The Netherlands (**Table 1; Supplementary table 1**).

4.2. DNA extraction, PCR, and sequencing

DNA was extracted for all isolates following the protocol described by De Beer et al. (2014). The DNA solutions were used as template for PCR and the remainder were stored at -20°C.

Three gene regions were chosen for amplification, sequencing, and phylogenetic analyses. The 28S ribosomal large subunit (LSU) region was amplified using the primers LR0R and LR5 (Vilgalys & Hester 1990), the ITS1-5.8S-ITS2 region (ITS) was amplified using the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990), and the partial beta-tubulin (*β-tubulin*) gene region was amplified using primers T10 (O'Donnell & Cigelnik 1997) and Bt2b (Glass & Donaldson 1995). PCR reactions were carried out as described by Duong et al. (2012) in a 25 µL reaction volume and the annealing temperature was 55°C.

PCR products were treated before sequencing using the ExoSAP-IT™ PCR Product Cleanup Reagent (ThermoFisher Scientific, Waltham, Massachusetts) following the manufacturer's protocols. Sequencing PCR reactions were performed for both forward and reverse primers as used in PCR. The samples were prepared for sequencing per the BIGDYE® TERMINATOR 3.1 Cycle sequencing protocol (ThermoFisher Scientific) using an annealing temperature of 55°C. Products were purified using sodium acetate and ethanol precipitation and submitted for sequencing on an ABI PRISM®3100 Genetic Analyzer (Applied Biosystems, Foster City, California).

4.3. Phylogenetic analyses

Consensus sequences were used for BLAST searches against the NCBI GenBank non-redundant nucleotide sequence database to identify primary taxonomic grouping of the isolates. Based on these groupings, a total of three datasets were prepared for phylogenetic analyses (one LSU, one ITS and one *β-tubulin*). Reference sequences for these datasets were selected based on the phylogenies of De Beer & Wingfield (2013), De Beer et al. (2016 a & b) and downloaded from NCBI GenBank (Treebase Project no: 28436). For the analyses of the *β-tubulin* dataset, the intron and exon regions were determined for the different isolates based on the *β-tubulin* gene map compiled by Yin et al. (2015).

Each dataset was analysed separately due to uneven sequence availability among the different datasets. Alignments were done using the online version of MAFFT 7 (Katoh & Standley 2013) using default settings. The datasets were treated with GBLOCKS 0.91b (Castresana 2000) using the less stringent options to eliminate poorly aligned positions before phylogenetic analysis. Maximum parsimony analyses were performed using MEGA 6.06 (Tamura et al. 2013). Phylogenies were generated using Subtree-Pruning-Regrafting algorithm starting with 10 random initial trees. Alignment gaps and missing data were included. Node confidence levels were tested using 1000 bootstrap replicates. Maximum likelihood analyses were performed using the software RAXMLGUI 2 (Silvestro & Michalak 2012, Elder et al. 2019). Phylogenies were generated following the GENERAL TIME REVERSIBLE + GAMMA (GTR+G) nucleotide substitution model. Ten parallel runs each with 1000 thorough bootstrap replicates were conducted. Bayesian inference analyses were performed using MRBAYES 3.2.5 (Ronquist & Huelsenbeck 2003). Phylogenies were generated following the MARKOV CHAIN MONTE CARLO (MCMC) method using the GTR+G model. Ten parallel runs, each with four independent MCMC chains were conducted. Trees were sampled every 100th generation for 5 million generations. Estimated sample size (ESS) and potential scale reduction factor (PSRF) values were used to confirm proper sampling and run convergence had been achieved. Trees sampled during the burn-in phase (25 % of trees sampled) were discarded and posterior probabilities were calculated from the remaining trees.

4.4. Genome sequencing

Due to incongruence between phylogenetic analyses of different gene regions and the unique ecology of the isolates obtained from *Termitomyces* combs, we explored the use of phylogenomic methods to clarify the placement of these species within the Ophiostomataceae. To this end, the genomes of two isolates (CMW 47056 and CMW 47058) each representing a newly identified major lineage (referred to as lineage B and A respectively in the analyses) were sequenced and assembled. Single-conidial isolates of cultures CMW 47056 and CMW 47058 were grown in McCartney bottles containing 10 mL malt yeast broth (YM: 2 % malt extract, 0.5 % yeast extract, Biolab, Midrand, South Africa). The mycelium was collected by centrifugation in 2 mL Eppendorf tubes and freeze dried. Genomic DNA was extracted from freeze-dried mycelium using the salt-based extraction method described by Duong

et al. (2013). A TruSeq PCR-Free paired-end library (550 bp average insert sizes) was prepared for each isolate and sequenced using the Illumina HiSeq2500 Platform (Macrogen Inc, Seoul, Korea) to obtain 251 bp paired-end reads. Quality and adapter trimming of pair-end reads was carried out using TRIMMOMATIC 0.36 (Bolger et al. 2014). *De novo* assembly of the genomes were carried out using SPADES 3.9 (Bankevich et al. 2012), followed by scaffolding using SSPACE-STANDARD 3.0 (Boetzer et al. 2011). Assembly gaps were filled or extended using GAPFILLER 1.10 (Boetzer & Pirovano 2012). The completeness of each assembly was assessed using the BENCHMARKING UNIVERSAL SINGLE-COPY ORTHOLOGS (BUSCO) 4.0.5 tool against the Fungi dataset (Seppey et al. 2019).

4.5. Taxon sampling and phylogenomic analyses

To improve our understanding on the phylogenetic placement of the putative new species originating from *Termitomyces* combs, we performed phylogenomic analyses using both supermatrix and supertree approaches on protein sequence data. Along with the two newly generated genome sequences, we included genome data for 32 species, representing 10 currently recognized genera in the Ophiostomatales and four outgroup taxa. All additional genomes utilized in this study were downloaded from the JGI fungal genome portal MycoCosm (http://jgi.doe.gov/fungi) and NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/) (**Table 2**).

All 34 genomes were assessed using BUSCO 4.0.5 tool against the Sordariomycota dataset (Seppey et al. 2019). Common single-copy BUSCO genes shared across all 34 genomes were identified and their amino acid sequences were extracted from the BUSCO run results. Individual gene datasets were compiled for each of the BUSCO genes and aligned with MAFFT 7.407 using the --auto option (Katoh & Standley 2013). After alignment, resulting datasets were trimmed using TRIMAL 1.4 using the -automated1 option (Capella-Gutiérrez et al. 2009). Datasets shorter than 100 amino acids after trimming were excluded from further analysis. Permutation Tail Probability (PTP) tests were conducted to identify datasets with no phylogenetic signal and those that resulted in a P-value > 0.01 were excluded from further analyses.

4.5.1. Supermatrix analyses

Datasets retained after filtering were concatenated into a single supermatrix using FASCONCAT 1.04 (Kück & Meusemann 2010). PARTITIONFINDER 2.1.1 was used to identify best partition scheme for the concatenated dataset (Lanfear et al. 2017). Maximum likelihood (ML) analysis was performed on the supermatrix using RAXML with 20 random ML searches followed by 100 rapid bootstrap replications under the PROTGAMMAJTT model and the partition scheme was specified according to the PARTITIONFINDER results (Stamatakis & Alachiotis 2010).

4.5.2. Supertree analyses

Since phylogenetic reconstruction using the concatenation approach can introduce bias due to genes with strong phylogenetic signal (Shen et al. 2017), two supermatrix approaches, namely ASTRAL (Mirarab et al. 2014) and STAG (Emms et al. 2018), were also used in the phylogenomic analyses. ASTRAL identifies quartet topologies from input gene trees and searches for the consensus tree that has the maximum number of quartet topologies (Mirarab et al. 2014). STAG uses a standard "greedy" consensus method to construct a concensus tree, using distance-based trees that were derived from the input gene trees (Emms et al. 2018). Individual gene trees were generated for all BUSCO datasets using IQ-TREE 2 (Minh et al. 2020) with 1000 ultrafast bootstraps and an optimal amino acid substitution model that was automatically determined (Hoang et al. 2018). The trees obtained from IQ-TREE were used directly as input trees for STAG analysis. For ASTRAL analysis, branches in gene trees with less than 30 % bootstrap support were collapsed before they were used for species tree construction. To compare the different phylogenomic trees obtained using the three approaches, tree certainty values were calculated with RAXML using the individual ML trees as input trees (Salichos & Rokas 2013). To estimate support for nodes in the species trees obtained using the three approaches, gene and site concordance factors were calculated in IQ-TREE 2 using the gene trees and the supermatrix respectively.

4.6. Species descriptions

4.6.1. Morphology

Ascomata were removed from sections of *Termitomyces* combs by soaking the material in 10 % KOH solution. The recovered ascomata were mounted on glass slides in 80 % lactic acid. To observe asexual structures for the various isolates, cultures were grown directly on the microscope slide using blocks of MEA. Cultures were maintained for up to 3 days at ambient room temperature before the agar blocks were removed, 80 % lactic acid was added to the structures left on the slide and then covered with a cover slip. Morphological observations were made using a Zeiss AxioScop2 microscope with a Zeiss Axiocam ICc5 camera attached (Carl Zeiss, Oberkochen, Germany). Measurements were taken for ascospores, conidia, conidiophores, conidiogenous cells, the ascomatal neck width (base and tip) and length, as well as some additional characteristics when present. Values were determined from measurements of 50 or more structures.

4.6.1. Growth study

A growth study was conducted to determine optimal growth conditions for the different lineages. Two isolates from each lineage were selected for the experiment. Agar plugs of 5 mm in diameter were excised from the actively growing edge of a 10 d old culture and placed mycelium side down onto the centre of a 90 mm MEA plate. Plates were incubated in the dark at a temperature range from 5–35°C at 5°C intervals. Colony diameter was measured daily for 10 d or until the mycelium reached the edge of the plates. Five replications were prepared for each of the isolates at each temperature treatment.

4.7. Growth on **Termitomyces** *associated media*

Selected isolates (**Table** 1) representing all taxa isolated from *Termitomyces* combs were grown on different media derived from the *Termitomyces* combs to determine whether compounds present in the material enhances their growth and/or sporulation.

4.7.1. Fungus comb enriched medium (FCE)

Dried *Termitomyces* comb was ground to a fine powder using a mortar and pestle. Ten grams (g) of the ground powder was suspended in 250 mL autoclaved distilled water. The bottle was placed on a rotary shaker for 6 h before the liquid was collected in 50 mL falcon tubes and centrifuged for 10 min at 8500 rpm. The supernatant was collected and sterilized using a 0.2 µm syringe filter. The filtration step was repeated once to ensure that the filtrate was free from microbial contaminants. A total of 150 mL of the filter-sterilized extract was added to 850 mL autoclaved MEA kept at 50°C to make up 1 L medium and immediately poured into Petri plates.

4.7.2. Termitomyces *extract medium (TE)*

Termitomyces sp. (LSU Accession: MT835246; ITS Accession: MT845206) cultures were grown in McCartney bottles containing 10 mL MY broth. Cultures were incubated for 2–3 wk after which the culture broth was collected by centrifugation at 8500 rpm for 10 min and sterilized using a 0.2 µm syringe filter. The filtration step was repeated once to ensure that the filtrate was free from microbial contaminants. A total of 150 mL of the broth was added to 850 mL autoclaved MEA kept at 50°C to make up 1 L medium and immediately poured into Petri dishes.

4.7.3. Termitomyces *mycelium medium (TM)*:

The *Termitomyces* mycelium collected from the previous step was freeze-dried. A total of 10 g of freeze-dried mycelium was collected and added to 1 L MEA and macerated into the molten medium using a kitchen stick blender. After maceration, the mixture was autoclaved for 20 min at 121°C to sterilize before dispensing into Petri dishes.

Two isolates of each of the three putative new species identified in the phylogenetic analyses (T**able** 1) were selected to be grown on the various media mentioned above, as well as MEA as a control. Agar plugs (5 mm diameter) were excised from the edges of actively growing cultures and transferred to the centres of 60 mm Petri dishes containing the different media. Three replicates plates were prepared for each isolate on each of the four media. The plates were incubated at 25°C and colony measurements

were taken every day for 10 d or until isolates covered the entire plate surface. Incubation was continued for an extended period (up to two months) to observe cultures for the formation of sexual structures or changes in growth characteristics.

4.8. Statistical analyses

To determine if the different media had a significant effect on the growth of the isolates, statistical analyses were performed on the colony growth measurements taken on day eight of the growth trial using R (R Core Team, 2017; https://www.R-project.org/). Initial analyses consisted of Shapiro-Wilk and Levene tests to determine normal distribution and homogeneity of variance of the collected data. As the data were not normally distributed or homogeneous, an attempt was made to adjust the data using various methods including square root, cube root, log and Tukey's ladder of power transformations and the removal of outliers. Despite these attempts, the data could not be transformed and therefore the Kruskal-Wallis rank sum test (Kruskal & Wallis 1952) and *ad-hoc* Dunn multiple comparisons test (Dunn 1964) were conducted because they do not require normal distribution or homogeneity of the data.

5. Results

5.1. Fungal isolation and preliminary identification

Isolations from ascomata on the *Termitomyces* combs yielded 49 isolates (**Table 1**). The LSU, ITS and *β-tubulin* regions were sequenced, and representatives were selected for inclusion in preliminary phylogenetic analyses. Single locus analysis of the LSU dataset (**Supplementary Figure 1**) separated the isolates obtained from the *Termitomyces* combs in two distinct clades (lineage A and B), separate from other species in the Ophiostomataceae. A representative isolate from each of these clades was selected for whole genome sequencing and inclusion in phylogenomic analyses.

5.2. Genome sequencing

Illumina sequencing of two isolates, one from lineage A (CMW 47058 – SAMN18275857) and one from lineage B2 (CMW 47056 – SAMN18275856), yielded 20 906 584 and 13 902 124 paired-end

reads respectively. Genome assembly using the Illumina data of isolate CMW 47056 (B) yielded an assembly of 874 scaffolds (≥500 bp in size), an N50 of 55.22 Kb and a genome size of approximately 26.3 Mb, which is within the size range known for other species of Ophiostomataceae (19.5 Mb *Ceratocystiopsis brevicormi* (Vanderpool et al. 2018) – 43.8 Mb *Hawksworthiomyces lignivorus* (Wingfield et al. 2017)). Genome assembly using the Illumina data of isolate CMW 47058 (A) yielded an assembly of 398 scaffolds (≥500 bp in size), an N50 of 139.18 Kb and a genome size of approximately 16 Mb, which is the smallest genome sequenced thus far for any species in the Ophiostomataceae. BUSCO assessment using the fungi dataset showed the genome assemblies to be mostly complete, with the assembly of isolate CMW 47056 (B) being 98.4 % and the assembly of isolate CMW 47058 (A) being 90.1 % complete.

5.3. Phylogenomic analyses

To clarify the placement of lineages A and B within the Ophiostomataceae, phylogenomic analyses were performed using whole genome sequence data from 34 species, 30 of which were species in the Ophiostomatales and four outgroup taxa. A total of 1879 shared single copy BUSCO genes were identified in the genomes of all 34 investigated taxa. Ten of these were found to be shorter than 100 amino acids after aligning and trimming and thus were removed from further analyses. The Permutation Tail Probability (PTP) test then identified four additional alignments that had no phylogenetic signal with a P-value > 0.01, and these were also removed from further analyses. Phylogenies obtained from supermatrix (RAXML) and supertree (ASTRAL and STAG) approaches differed in the placement of isolates CMW47056 (B) and CMW 47058 (A) representing two of the lineages identified from termite fungus combs within the larger Ophiostomataceae (**Figure 1**). Based on maximum likelihood scores, the phylogeny obtained from RAXML analysis scored as the best of the phylogenies obtained, whereas tree certainty measures indicated that the phylogeny obtained from STAG analysis had the highest level of certainty. The RAXML phylogeny grouped isolates CMW47056 (B) and CMW 47058 (A) as sister taxa and both formed a sister clade to *Ophiostoma*. The STAG phylogeny had a similar grouping of isolates CMW47056 (B) and CMW 47058 (A), but together they formed a sister clade to both *Ophiostoma* and *Sporothrix*. The ASTRAL phylogeny grouped CMW47056 (B) as a sister taxon to *Ophiostoma* and CMW

47058 (A) as sister taxon to both *Ophiostoma* and *Sporothrix*. Other than the conflicting placement of isolates CMW47056 (B) and CMW 47058 (A) and that of *Fragosphaeria purpurea* and *Graphilbum fragrans*, the placement of the remaining investigated species and genera in the Ophiostomataceae was consistent in all phylogenomic trees.

5.4. Phylogenetic analyses

Single locus phylogenetic analyses the of ITS and *β-tubulin* regions were used to determine species-level taxonomic assignment of isolates. Isolates of lineage A formed a single, well-supported clade in both the LSU and ITS analyses (**Figure 2A; Supplementary Figure 1**). Despite numerous attempts using different temperatures and primer pairs, we were unable to amplify the *β-tubulin* region for isolates in lineage A. Isolates of lineage B formed a single clade in the LSU analyses (**Supplementary Figure 1**) but formed two well supported sub-clades (lineage B1 and lineage B2) in both the ITS and *β-tubulin* analyses (**Figure 2 A & B**). Using the *β-tubulin* gene map compiled by Yin et al. (2015) we determined that lineage B1 has an intron arrangement of 2/-/-/5 and lineage B2 an arrangement of 2/3/-/5. The distinct intron arrangements for lineage B1 and B2, along with morphological differences between isolates residing in the two lineages discussed in the Taxonomy section, provides support for their separation as two distinct species.

5.5. Taxonomy

Based on phylogenetic and phylogenomic analyses, the isolates from *Termitomyces* combs separated into three lineages (lineages A, B1 and B2), each distinct from each other and from all other known species in the Ophiostomataceae. Based on their unique niche and phylogenetic placement within the various analyses, two new genera are introduced in the Ophiostomataceae to accommodate the three new species revealed by the different lineages.

5.5.1. Lineage A

Chrysosphaeria W.J. Nel, Z.W. De Beer, T.A. Duong gen. nov.

MycoBank no: 837564

Etymology: From Latin chryso-, golden, and -sphaera, sphere or orb, referring to the light colour of the ascoma bases in the type species.

Diagnosis: Distinguished from all other related genera of the Ophiostomatales due the unique morphology of the ascomata. Throughout their development, these structures remain golden brown in colour and the ascomatal necks retain their flexibility (i.e. ability to bend in different directions without breaking).

Type species: ***Chrysosphaeria jan-nelii*** W.J. Nel, Z.W. De Beer & T.A. Duong sp. nov. **Figure 3**

MycoBank no: 837566

Typification: SOUTH AFRICA. LIMPOPO PROVINCE: Mookgophong, Amsterdam farm, ascomata embedded in *Termitomyces* fungal comb of *M. natalensis*, Feb 2015, *W.J. Nel* (**holotype** PREM 63088). Living ex-type culture CMW 47058 = CBS 141570. GenBank: LSU= MT637006; ITS = MT637038; *β-tubulin* = MT649128.

Etymology: named for the late Professor J. J. C. Nel (1933-2019), a South African entomologist who worked on termites, grandfather of the first author and from whom she drew significant inspiration.

Description: Sexual state. Ascomatal bases in culture light to golden brown, globose, (108–)129–171(–189) µm in diameter, ornamented with short hyaline to lightly pigmented hairs. *Ascomatal necks* light brown, tapering and becoming hyaline towards apex, flexible, (309–)502–771(–989) µm long, (28–)35–48(–56) µm wide at base, (11–)13–18(–22) µm wide at apex. *Ostiolar hyphae* present, slightly divergent, hyaline. *Asci* not seen. *Ascospores* produced in slimy droplet at apex of neck, hyaline, short cylindrical to bean shaped, aseptate, no sheath, (3.0–)3.5–4.5(–5.0) µm x (1.0–)1.5–2 µm. *Asexual state. Sporothrix*-like. *Conidiophores* hyaline, micronematous, (24–)38–85(–104) µm long. *Conidiogenous cells* denticulate, hyaline, (5–)11–39 µm. *Primary conidia* hyaline, aseptate, oblong, (9.0–)10.0–12.5(–14.5) µm x (3.0–)3.5–4.0(–4.5) µm. *Secondary conidia* hyaline, obovoid, aseptate. *Culture characteristics*: Colonies hyaline with white aerial mycelium on MEA, mycelium mostly superficial on agar, slow growing, growing on average 40 mm in 10 d at 25°C, grows best at 25°C, no growth at 35°C. Protoperithecia sometimes visible after 1–2 mo in culture.

Additional specimens examined: SOUTH AFRICA. LIMPOPO PROVINCE: Mookgophong, Amsterdam farm, ascomata embedded in *Termitomyces* fungal comb of *M. natalensis*, Feb 2015, *W.J.*

Nel (**paratype** PREM 63087). Living culture CMW 47057 = CBS 141566. GenBank: LSU= MT637008; ITS = MT637037. SOUTH AFRICA. LIMPOPO PROVINCE: Mookgophong, Amsterdam farm, ascomata embedded in *Termitomyces* fungal comb of *M. natalensis*, Feb 2015, *W.J. Nel* (**paratype** PREM 63089). Living culture CMW 46495 = CBS 141571. GenBank: LSU= MT637007; ITS = MT637042. SOUTH AFRICA. LIMPOPO PROVINCE: Mookgophong, Amsterdam farm, ascomata embedded in *Termitomyces* fungal comb of *M. natalensis*, Feb 2015, *W.J. Nel*. Living culture CMW 48039. GenBank: LSU= MT637009; ITS = MT637036.

Notes: Multiple attempts to amplify *β-tubulin* gene region from *C jan-nelii* were unsuccessful. A *β-tubulin* gene was identified from the genome sequence using a tBLASTx against the reference *β-tubulin* sequence from *Intubia oerlemansii*. Sequence comparison revealed that the *β-tubulin* gene of this species is highly divergent from other species of Ophiostomataceae. Identification of introns based on *β-tubulin* map compiled by Yin et al. (2015) showed that *C. jan-nelii* also has an intron arrangement 2/3/-/5 similar to that in isolates of *I. oerlemansii.*

5.5.2 Lineage B

Intubia W.J. Nel, Z.W. De Beer, T.A. Duong gen. nov.

MycoBank no: 837565

Etymology: From the Xhosa language, Intubi for termite, recognising the source where the fungus was found.

Diagnosis: Distinguished from other phylogenetically related genera in the Ophiostomatales for their unique habitat with dark coloured, ascomata embedded in the substrate of termite abandoned *Termitomyces* combs

Lineage B1

Type species: ***Intubia macrotermitinarum*** W. J. Nel, Z. W. De Beer, T. A. Duong sp. nov. **Figure 4**

Mycobank no: 837567

Typification: SOUTH AFRICA. GAUTENG PROVINCE: Pretoria, Rietondale, Plant protection research institute, ascomata embedded in *Termitomyces* fungal comb of *M. natalensis*, Feb 2015, *W.J.*

Nel (**holotype** PREM 63090). Living ex-type culture CMW 46496 = CBS 141560. GenBank: LSU = MT636994; ITS = MT637025; BT = MT649132.

Etymology: named refers to the termite sub-family *Macrotermitinae* to which all species of fungus-growing termites belong.

Description: Sexual state. Ascomatal bases dark brown, globose (117–)153–250(–302) µm in diameter. *Ascomatal necks* uniformly dark, often slightly curved, tapering towards apex, (1314–)745–2723(–3689) µm long, (26–)40–62(–80) µm wide at base, (10–)15–21(–25) µm wide at apex. *Ostiolar hyphae* absent. *Asci* not seen. *Ascospores* produced in slimy droplet at apex of the neck, hyaline, cylindrical, sometimes slightly curved, aseptate, no sheath, (5–)5.5–6.5(–7.0) µm x 1.5–2.0 µm. *Asexual state*. *Hyalorhinocladiella*-like. *Conidiophores* hyaline, smooth, arising singly from hyphae, micronematous, 19–67(–143) µm long. *Conidiogenous cells* smooth, (11–)18–45(–98) µm long. *Conidia* hyaline, bacilliform tapering toward one end, (6.5–)7.0–9.5(–11.0) µm x (2.0–)2.5–3.0 µm wide at thickest part, aseptate, arising from conidiophore or directly from hyphae. Secondary conidia present. *Culture characteristics:* Colonies hyaline to whitish on MEA, mycelium superficial on agar with little aerial mycelium, slow growing, growing on average 39 mm in 10 d at 25°C, grows best at 30°C growing on average 58.6 mm in 10 d. Ascomata not observed in culture.

Additional specimens examined: SOUTH AFRICA. GAUTENG PROVINCE: Pretoria, Hatfield, University of Pretoria experimental farm, ascomata embedded in *Termitomyces* fungal comb of *M. natalensis*, Feb 2015, *W.J. Nel* (**paratype** PREM 63091). Living culture CMW 46492 = CBS 141561. GenBank: ITS = MT637030; *β-tubulin* = MT649129. SOUTH AFRICA. GAUTENG PROVINCE: Limpopo, Mookgophong, Amsterdam farm, ascomata embedded in *Termitomyces* fungal comb of *M. natalensis*, Feb 2015, *W.J. Nel*. Living culture CMW 47067 = CBS 141562. GenBank: LSU = MT645589; ITS = MT637049; *β-tubulin* = MT649163. SOUTH AFRICA. GAUTENG PROVINCE: Pretoria, Hatfield, University of Pretoria experimental farm, ascomata embedded in *Termitomyces* fungal comb of *M. natalensis*, Feb 2015, *W.J. Nel.* Living culture CMW 46494. GenBank: ITS = MT637061; *β-tubulin* = MT649121.

Lineage B2

Intubia oerlemansii W.J. Nel, Z.W. De Beer & T.A. Duong sp. nov. **Figure 5**

Mycobank no: 837568

Typification: SOUTH AFRICA. GAUTENG PROVINCE: Pretoria, Rietondale, Plant protection research institute, ascomata embedded in *Termitomyces* fungal comb of *M. natalensis*, Feb 2015, *W.J. Nel* (**holotype** PREM 63094). Living ex-type culture CMW 47048 = CBS 141564. GenBank: ITS = MT637024, BT = MT649147.

Etymology: name honours Mr. Arien Oerlemans, owner of the farm, Amsterdam, near Mookgophong, Limpopo Province, South Africa where many of the fungus combs were collected.

Diagnosis: Intubia oerlemansii differs from the other species in the genus in that it produces two conidial forms. There is also a difference in the intron/exon regions of the *β-tubulin* gene with *I. oerlemansii* having exon three and five present and *I. macrotermitinarum* having only exon five present.

Description: Sexual state. Ascomatal bases dark brown to black, globose, (109–)135–192(–233) µm diameter. *Ascomatal necks* dark brown, uniformly dark throughout, (1137–)1393–1900(–2198) µm long, tapering towards apex, (30–)36–49(–60) µm wide at base, (8.0–)10.0–14.5(–17.5) µm wide at apex. *Ostiolar hyphae* absent. *Asci* not seen. *Ascospores* produced in slimy droplet at apex, hyaline, cylindrical, occasionally slightly curved, aseptate, no sheath, (5.0–)5.5–6.5(–7.0) µm x 1.0–1.5(–2.0) µm. *Asexual state. Sporothrix*-like. *Conidiophores* hyaline, smooth, arising singly, micronematous, (9–)13–74(–135) µm long. *Conidiogenous cells* (8–)19–34(–45) µm long, denticulate. *Conidia* of two types. *Type 1:* formed on hyphae, hyaline, round to obovoid, aseptate, (3.5–)4.5–5.5(–6.5) µm x (2.5–)3.0–4.0(–5.0) µm. *Type 2:* formed on conidiophores, hyaline, bacilliform, aseptate, (5.0–)6.0–7.5(–8.5) µm x (1.5–)2.0–3.0(–3.5) µm, secondary conidia present. *Culture characteristics*: Colonies hyaline to whitish on MEA, mycelium superficial on agar, little aerial mycelium present, slow growing, growing on average 36 mm in 10 d at 25°C, grows best at 30°C growing on average 50.4 mm in 10 d. Ascomata not observed in culture.

Additional specimens examined: SOUTH AFRICA. GAUTENG PROVINCE: Pretoria, Rietondale, Plant Protection Research Institute, ascomata embedded in *Termitomyces* fungal comb of *M. natalensis*,

Feb 2015, *W.J. Nel* (**paratype** PREM 93095). Living culture CMW 47056 = CBS 141565. GenBank: LSU = MT637001; ITS = MT637034, BT = MT708983. SOUTH AFRICA. GAUTENG PROVINCE: Pretoria, Rietondale, Plant Protection Research Institute, ascomata embedded in *Termitomyces* fungal comb of *M. natalensis*, Feb 2015, *W.J. Nel* (**paratype** PREM 63098). Living culture CMW 46498 = CBS 141563. GenBank: LSU = MT940646; ITS = MT637040, BT = MT649134. SOUTH AFRICA. GAUTENG PROVINCE: Pretoria, Rietondale, Plant Protection Research Institute, ascomata embedded in *Termitomyces* fungal comb of *M. natalensis*, Feb 2015, *W.J. Nel*. Living culture CMW 47052. GenBank: LSU = MT637002; ITS = MT637029, BT = MT649151.

5.6. Growth on Termitomyces *associated media*

Chrysosphaeria jan-nelii: Analyses of growth rates on various media (MEA, FCE, TE, TM) using the Kruskal-Wallis rank sum test showed a significant difference in the growth on the supplemented media compared to the MEA controls for all isolates. Isolates of *C. jan-nelii* grew most rapidly on both MEA and FCE media and isolate CMW 47058 of this species had completely covered the surface of the FCE plates after eight days and, when growth measurements were assessed (**Figure 6**).

There was no significant difference (Dunn's test) in the growth rate of isolate CMW 47058 on MEA vs. FCE, but a marked increase in average growth on FCE was observed for both *C. jan-nelii* isolates growing, on average, at least 10 mm more than any of the isolates investigated. Isolates of *C. jan-nelii* also produced more aerial mycelium on all supplemented media tested (**Supplementary Figure 2**). Fully developed perithecia were observed for both isolates after 18 d incubation on the FCE media. Protoperithecia was observed on MEA plates but their formation usually took between 1–2 mo and did not progress beyond this stage.

Intubia: Isolates of *I. macrotermitinarum* and *I. oerlemansii* had a somewhat slower growth rates compared those of *C. jan-nelii*. A significant decrease in growth rate was noted on TM media compared to the MEA control for all four isolates tested. As was observed for the isolates of *C. jan-nelii*, an increase in aerial mycelium formation was noted in both species on FCE medium (**Supplementary Figure 2**).

6. Discussion

In this study, the taxonomic placement of 49 ophiostomatalean isolates growing on *Termitomyces* combs collected from the nests of fungus-growing *M. natalensis* termites was considered. Single locus phylogenetic analyses of sequence data generated for the rDNA 28S and ITS regions, along with that of the partial *β-tubulin* gene, grouped the isolates in three distinct lineages, which were not closely related to any other species in the Ophiostomataceae. Due to uncertainty regarding the generic placement of the two main clades in the phylogeny based on conventional gene sequencing, the genome of a single isolate residing in each of these clades was sequenced to consider their generic placement in the Family. Phylogenomic analyses including the genomes of 28 representatives of the Ophiostomatales, along with two newly sequenced isolates, confirmed their placement as two distinct lineages in the Ophiostomataceae. Consequently, the two novel genera, *Chrysosphaeria* and *Intubia*, were described to accommodate them. Three novel species, *Chrysosphaeria jan-nelii*, *Intubia macrotermitinarum*, and *I. oerlemansii*, were described in the new genera to accommodate all 49 isolates considered in this study.

Phylogenetic analyses using DNA sequences generated for three different gene regions (LSU, ITS and *β-tubulin*) showed that the genera *Chrysosphaeria* and *Intubia* reside in the Ophiostomataceae, but their placement in the Family was not clear. Uncertainty regarding appropriate generic placement, such as that found in this study, has been observed for other Ophiostomatalean genera including *Hawksworthiomyces* (De Beer et al. 2016b) and *Aureovirgo* (Van der Linde et al. 2016). Although the LSU and ITS regions are commonly used in phylogenetic analyses due to their ease of amplification and availability of reference sequence data (Schoch et al. 2012), these gene regions can be problematic in resolving higher level taxonomic relationships (Stielow et al. 2015). The availability of whole genome sequence data for a number of Ophiostomatalean species residing in a variety of different genera allowed us to adopt a phylogenomic approach to gain insights regarding the generic placement of the new genera in the Family. The decline in genome sequencing cost and the development of less computationally demanding phylogenomic analysis approaches will, in future, improve our ability to

appropriately define the limits of genera and species, as has recently been shown in *Fusarium* (Geiser et al. 2020), and in the present study.

Phylogenomic analyses using supertree and supermatrix approaches yielded conflicting results regarding the placement of *Chrysosphaeria* and *Intubia* in the larger Ophiostomataceae. Despite these incongruencies, both approaches suggested that the two genera were most closely related to, but distinct from *Ophiostoma* and *Sporothrix*. Both RAXML and STAG analyses indicated that *Chrysosphaeria* and *Intubia* are sister genera, suggesting a single origin of association with fungus-growing termites. In the RAXML phylogeny, both genera grouped as sister to *Ophiostoma*, whereas in the STAG phylogeny both genera were grouped as sister to both *Ophiostoma* and *Sporothrix*. In the ASTRAL analysis, *Intubia oerlemansii* (CMW47056) grouped as a sister taxon to *Ophiostoma* while *Chrysosphaeria jan-nelii* (CMW47058) grouped as a sister taxon basal to *Intubia*, *Ophiostoma* and *Sporothrix*. When considering the gene and site concordance factors as statistical support, the placement of *Chrysosphaeria* and *Intubia* in the STAG analysis was better supported than those from RAXML and ASTRAL.

The uncertainty regarding the generic placement was also seen for *Fragosphaeria purpurea* and *Graphilbum fragrans* when using the different analytical approaches. This problem was also observed in the phylogenomic study considering the Ophiostomatales by Vanderpool et al. (2018). Other than the uncertain placements mentioned above, the grouping of the remaining genera was consistent using the three approaches, as was also true in the phylogeny of Vanderpool et al (2018). Although we were not able to provide absolute confidence in the taxonomic placement of *Chrysosphaeria* and *Intubia*, phylogenomic analyses provided deeper insights into their position in the Ophiostomatales. If the taxonomic placements of the two new genera had relied solely on phylogenetic analyses of the LSU and ITS gene regions, *C. jan-nelii*, *I. macrotermitinarum* and *I. oerlemansii* would have been incorrectly treated as species in *Ophiostoma s.l.*

Because none of the new species identified in this study grouped with other species in the Ophiostomatales and had originated from a unique niche, growth factors relating to their termite association were considered. The fungi were shown to grow more actively on media supplemented with material from their termite associations than on control plates. Likewise, extended incubation of these

isolates on media supplemented with extracts from the fungal combs resulted in formation of mature ascomata, which did not occur on any other growth medium.

Termites are actively involved in tending their monoculture *Termitomyces* combs, maintaining them free of invasive "weedy" microbial species (Moriya et al. 2005; Otani et al. 2019). Soon after the death of a colony or artificial removal of the termites from the combs, *Xylaria* and *Pseudoxylaria* spp. rapidly overgrow the *Termitomyces* combs (Visser et al. 2009, 2011). The same phenomenon was observed with the new taxa described in this study, which only emerged once the termites were no longer present. However, the origin of the inoculum of these species remains to be discovered. They could occur in the soil before construction of the termite mound or alternatively might be acquired in the foraged material brought into the mound. It is also possible that they could be associates of other fauna such as mites, which are known to be associated with termites (Eickwort 1990; Wang et al. 2002), and have been shown to have close associations with other Ophiostomatalean fungi (Hofstetter et al. 2013; Chang et al. 2020). In this regard, the discovery of two new genera of fungi closely associated with the biology of termites exposes a fascinating new area of investigation that deserves to be pursued.

7. Conclusions

Ophiostomatalean fungi were discovered for the first time on the *Termitomyces* fungus combs of fungus-growing termites. Phylogenetic and phylogenomic analyses showed that these fungi represent three species residing in two novel genera of Ophiostomataceae. The Ophiostomatalean species discovered in this study appear to have had a long and specific association with termites. This emerged from the results of the phylogenomic analyses and was also consistent with their preferential growth on media containing extracts of the termite-fungus combs. These fungi appear only after termites have abandoned their combs, which is similar to other fungi that have been discovered in this unique niche. The role of these fungi in the biology of the termites or their associates remains to be explored.

8. Acknowledgements

We thank Dr. Seonju Marincowitz for assistance with microscopy. This project was funded by The National Research foundation of South Africa (NRF), The University of Pretoria, the Tree Protection Co-operative Programme (TPCP) and DSI-NRF Centre of Excellence in Plant-Health Biotechnology (CPHB), and the DSI-NRF SARChI Chair for Fungal Genomics.

Table1. Fungal isolates obtained and used in phylogenetic analysis in this study

Species	Isolate number			Geographical Origin	Host	GenBank accession numbers		
	CMW	CBS	PREM			ITS	LSU	*β-tubulin*
Lineage A:	48039			Amsterdam farm, Mookgophong, Limpopo, SA	Fungus comb from termite mounds	MT637036	MT637009	
Chrysosphaeria jan-nelii								
	47057	141566	63087	Amsterdam farm, Mookgophong, Limpopo, SA	Fungus comb from termite mounds	MT637037	MT637008	
	47058[a]	**141570**	**63088**	Amsterdam farm, Mookgophong, Limpopo, SA	Fungus comb from termite mounds	MT637038	MT637006	MT649128
	46495[a]	141571	63089	Amsterdam farm, Mookgophong, Limpopo, SA	Fungus comb from termite mounds	MT637042	MT637007	
Lineage B1:	46492[a]	141561	63091	University of Pretoria, Experimental farm, Pretoria, SA	Fungus comb from termite mounds	MT637030		MT649129
Intubia macrotermitinae	46494			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637061		MT649121
	46496[a]	**141560**	**63090**	PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637025	MT636994	MT649132
	47066			Amsterdam farm, Mookgophong, Limpopo, SA	Fungus comb from termite mounds	MT637048	MT636993	MT649162
	47067	141562		Amsterdam farm, Mookgophong, Limpopo, SA	Fungus comb from termite mounds	MT637049	MT645589	MT649163
	47068			Amsterdam farm, Mookgophong, Limpopo, SA	Fungus comb from termite mounds	MT637050		MT649164
	48941			South Africa	Fungus comb from termite mounds	MT835247		MW002071
Lineage B2:	46498[a]	141563	63098	PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637040	MT940646	MT649134
Intubia oerlemansii	47036			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637010		MT649135
	47048	**141564**	**63094**	PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637024		MT649147
	47052			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637029	MT637002	MT649151
	47056[a]	141565	63096	PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637034	MT637001	MT708983
	47071			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637063		MT649167
	48942			South Africa	Fungus comb from termite mounds	MT835248		MW002072

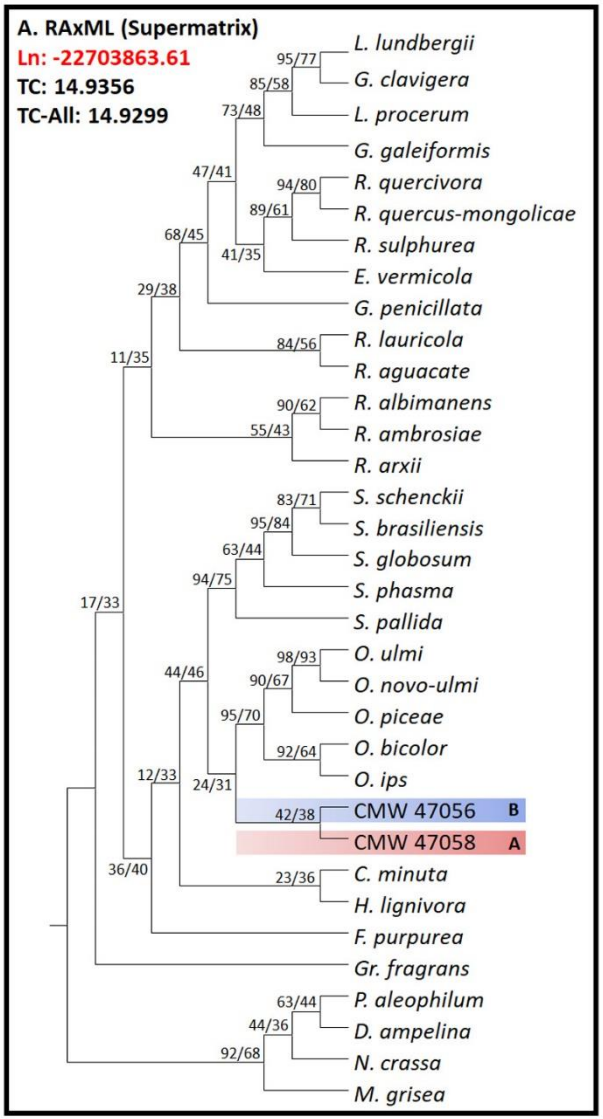
[a]Isolates used in growth studies

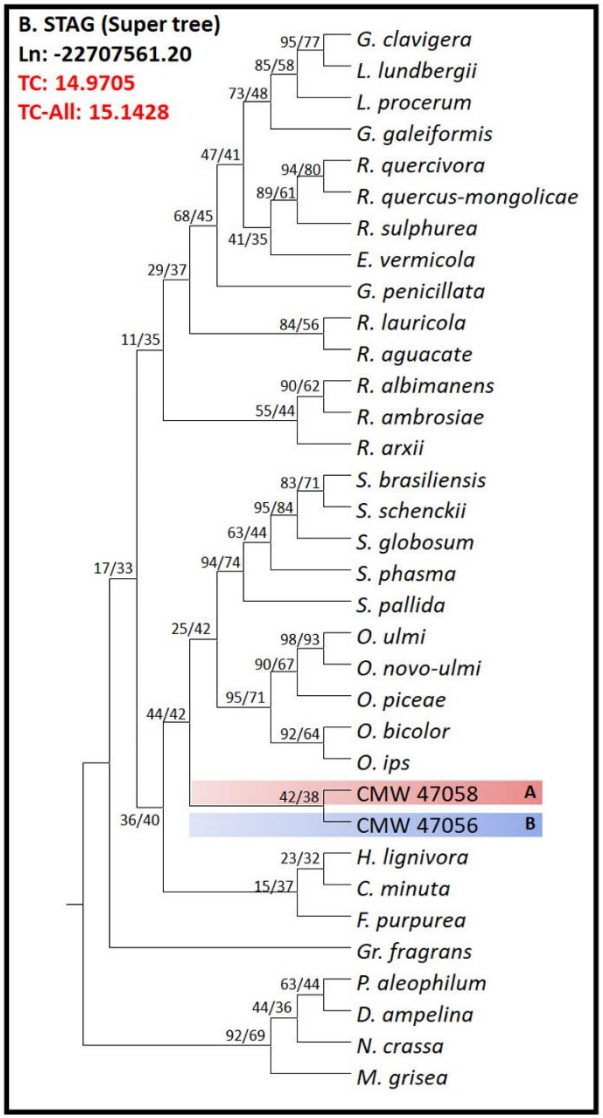
Bold face = ex-type isolates

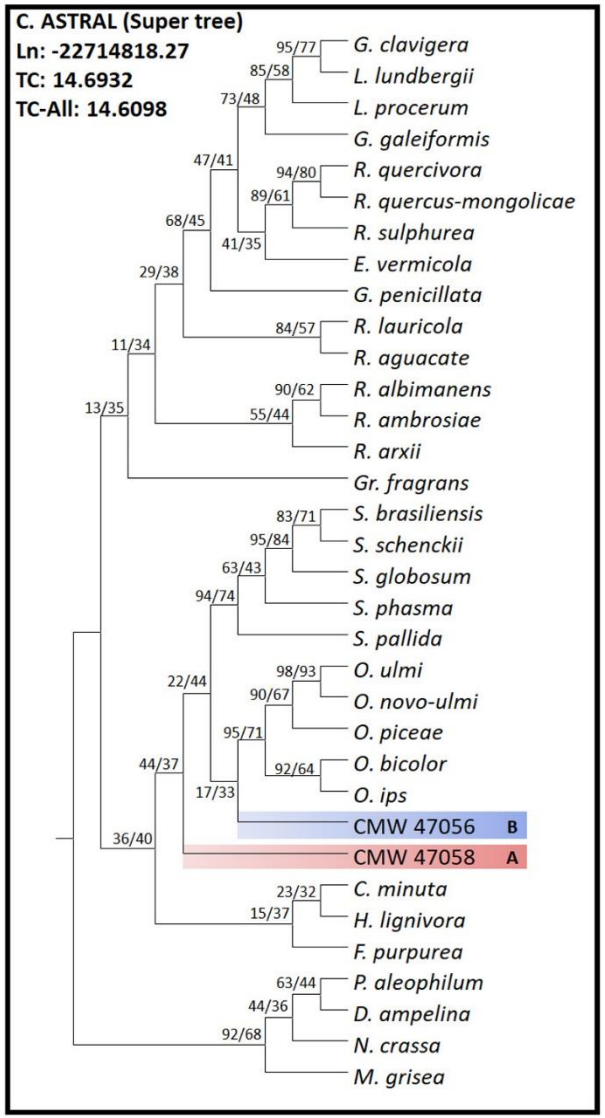
Table2. Isolates used in genomic analyses

Species name	Collection number	Origin	Size (Mb)	Reference
Ceratocystiopsis minuta	CMW 4352	*Ips cembrae*	21.2	Wingfield et al. 2016a
Diaporte ampelina	DA912	Grapevine	50.0	Morales-Cruz et al. 2015
Esteya vermicola	CBS 115803	*Scolytus intricatus*	34	Vanderpool et al. 2018
Fragosphaeria purpurea	CBS 133.34	*Fagus sylvatica*	34.8	Vanderpool et al. 2018
Grosmannia clavigera	UAMH11150	*Dendroctonus ponderosae*	29.1	DiGuistini et al. 2011
Graphilbum fragrans	CBS 138720	*Hylastes angustatus*	34.3	Wingfield et al. 2015b
Grosmannia galeiformis	CBS 115711	*Pinus sylvestris* infested with *Tomicus piniperda*	26.4	Wingfield et al. 2018b
Grosmannia penicillata	CBS 134055	*Ips amitinus*	26.3	Wingfield et al. 2016b
Hawksworthiomyces lignivorus	CMW 18600	Eucalyptus pole at soil level	43.8	Wingfield et al. 2017
Leptographium lundbergii	CMW 2190	*Pinus sylvestris*	26.5	Wingfield et al. 2015a
Leptographium procerum	CMW 34542	*Pinus resinosa*	28.5	Van der Nest et al. 2014b
Magnaporthe grisea	70-15	Rice pathogenic strain	41.5	Dean et al. 2005
Neurospora crassa	OR74A	Inbred genetically stable reference wild-type strain	41	Galagan et al. 2003
Ophiostoma bicolor	ZLVG358	*Picea abies*	25	Lah et al. 2017
Ophiostoma ips	CMW 19371	*Pinus taeda*	26	Wingfield et al. 2017
Ophiostoma novo-ulmi	H327	Elm	31.8	Forgetta et al. 2013
Ophiostoma piceae	UAMH 11346	*Pinus contorta*	32.5	Haridas et al. 2012
Ophiostoma ulmi	-	-	31.1	Khoshraftar et al. 2013
Phaeoacremonium aleophilum	UCR-PA7	Margin of grapevine (*Vitis vinifera* cv. “Thomson”)	47.4	Blanco-Ulate et al. 2013
Raffaelea aguacate	R272	*Persea americana*	35.2	Vanderpool et al. 2018
Raffaelea albimanens	CBS 271.70	*Platypus externedentatus* in *Ficus sycomo*	39	Vanderpool et al. 2018
Raffaelea ambrosiae	CBS 185.64	*Platypus cylindrus* gallery in *Quercus*	40.5	Vanderpool et al. 2018
Raffaelea arxii	CBS 273.70	*Xyleborus torquatus* on *Cussonia umbellif*	36.7	Vanderpool et al. 2018
Raffaelea lauricola	R570	Unknown	34.3	Vanderpool et al. 2018
Raffaelea quercivora	CBS 122982	Discolored sapwood of dead *Quercus mongolica*	25.9	Masuya et al. 2016
Raffaelea quercus-mongolicae	KACC44405	*Platypus koryoensis* infested *Quercus mongolica*	27	Jeon et al. 2017
Raffaelea sulphurea	CBS380.68	*Xyleborus saxesenii* gallery in *Populus deltoides*	23.2	Vanderpool et al. 2018
Sporothrix brasiliensis	ATCC MYA-4823	Feline skin lesion	33.2	Teixeira et al. 2014
Sporothrix globosa	CBS 120340	Face lesion	33.5	Huang et al. 2016
Sporothrix pallida	SPA8	Soil	37.8	D'Alessandro et al. 2016
Sporothrix phasma	CBS 119721	*Protea laurifolia*	30.2	Liu et al. 2019
Sporothrix schenckii	ATCC MYA-4821	Patient manifesting subcutaneous sporotrichosis	32.4	Teixeira et al. 2014
Chrysosphaeria jan-nelii	CMW 47058	*Termitomyces* fungus comb	16	This study
Intubia macrotermitinae	CMW 47056	*Termitomyces* fungus comb	26.3	This study

Figure 1. Phylogenomic trees obtained using A. supermatrix (RAxML) and B & C. supertree (ASTRAL and STAG) approaches. Trees were constructed from 1865 shared single copy BUSCO genes present in all taxa investigated. Ln: maximum likelihood score. TC: tree certainty. TC-All: tree certainty including all conflicting bipartitions. Concordance factors determined using IQ-TREE 2 are presented at nodes as gene concordance factor/site concordance factor (gCF/sCF).







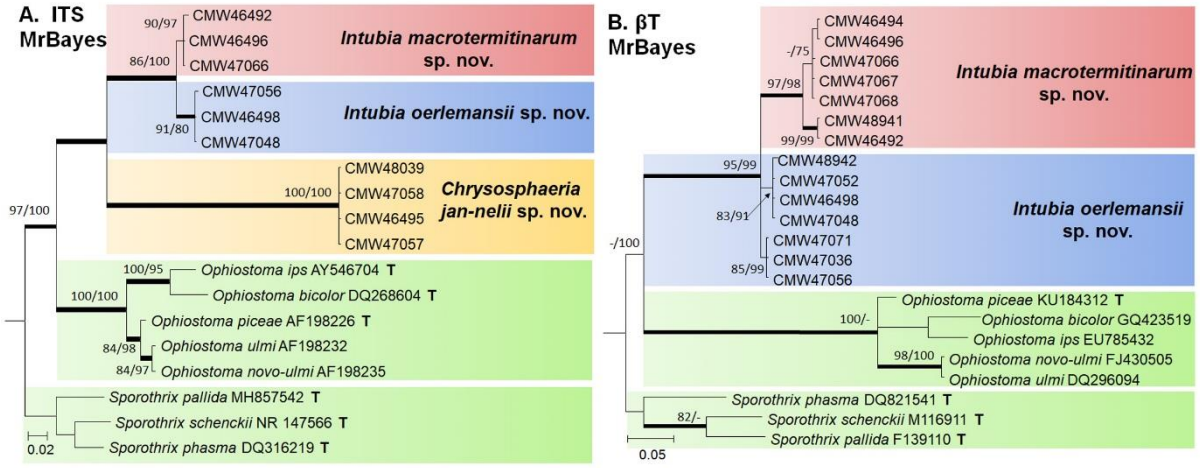


Figure 2. Bayesian inference trees derived from the analysis of A. the internal transcribed spacer and B. *β-tubulin* datasets. Bold black bands indicate posterior probabilities of 1.0, maximum likelihood (ML) and maximum parsimony (MP) bootstrap values above 75%, are indicated as ML/MP. – indicates an unsupported node in an analysis T = ex-type isolates

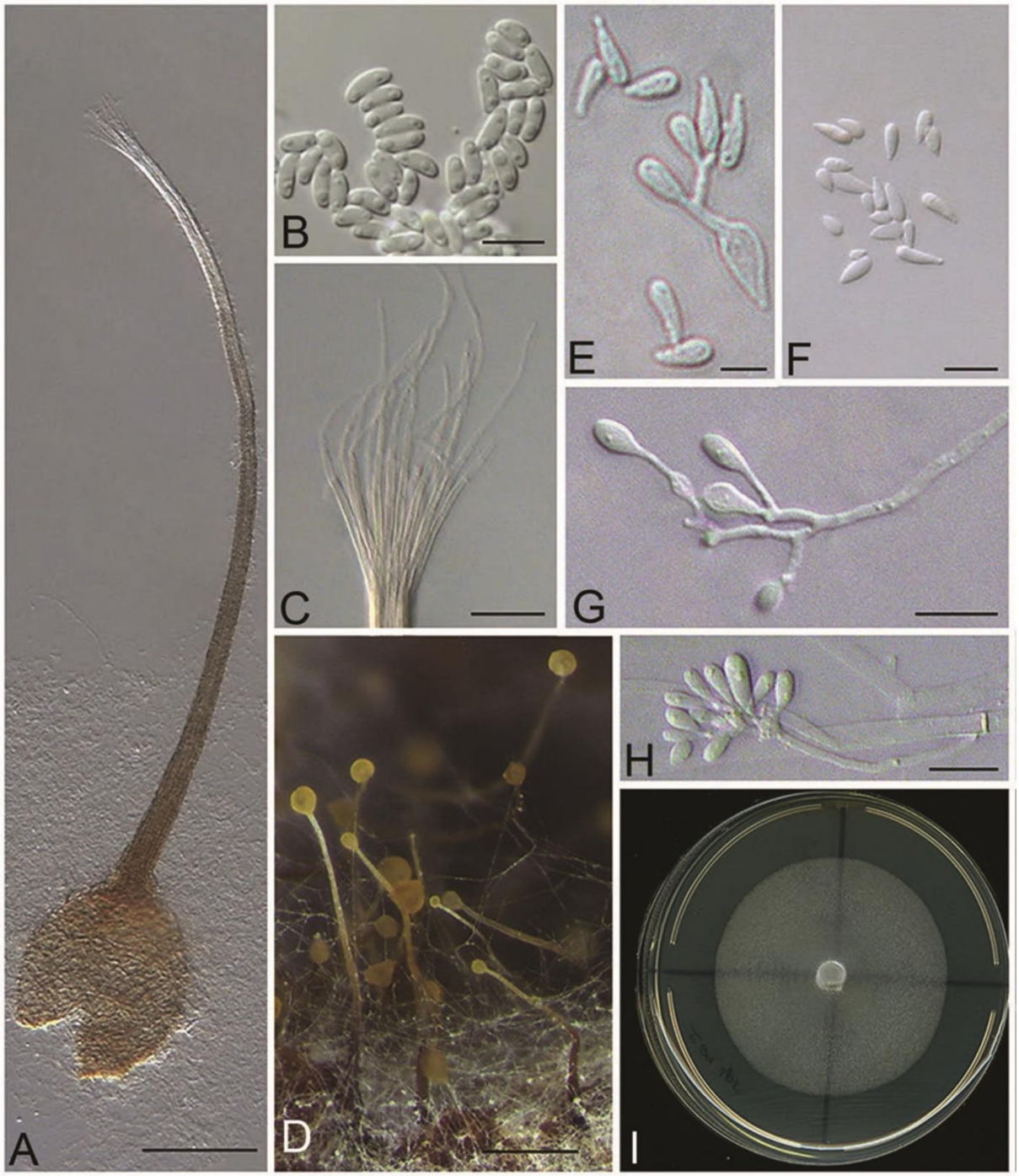


Figure 3. Morphological characteristics of the sexual and asexual structures of *Chrysosphaeria jannelii* sp. nov. (CMW 47058). A. Long-necked ascoma; B. Ascospores C. Hyaline apex with ostiolar hyphae; D. Ascomata on termite fungus comb with ascospores in slimy droplets; E. Mature primary conidia giving rise to secondary conidia; F. Primary conidia; G & H. Conidiogenous cell with conidia; I. pure culture on MEA. Bar: D = 200 µm; A = 100 µm; C = 20 µm; F, G, H = 10 µm; B, E = 5 µm

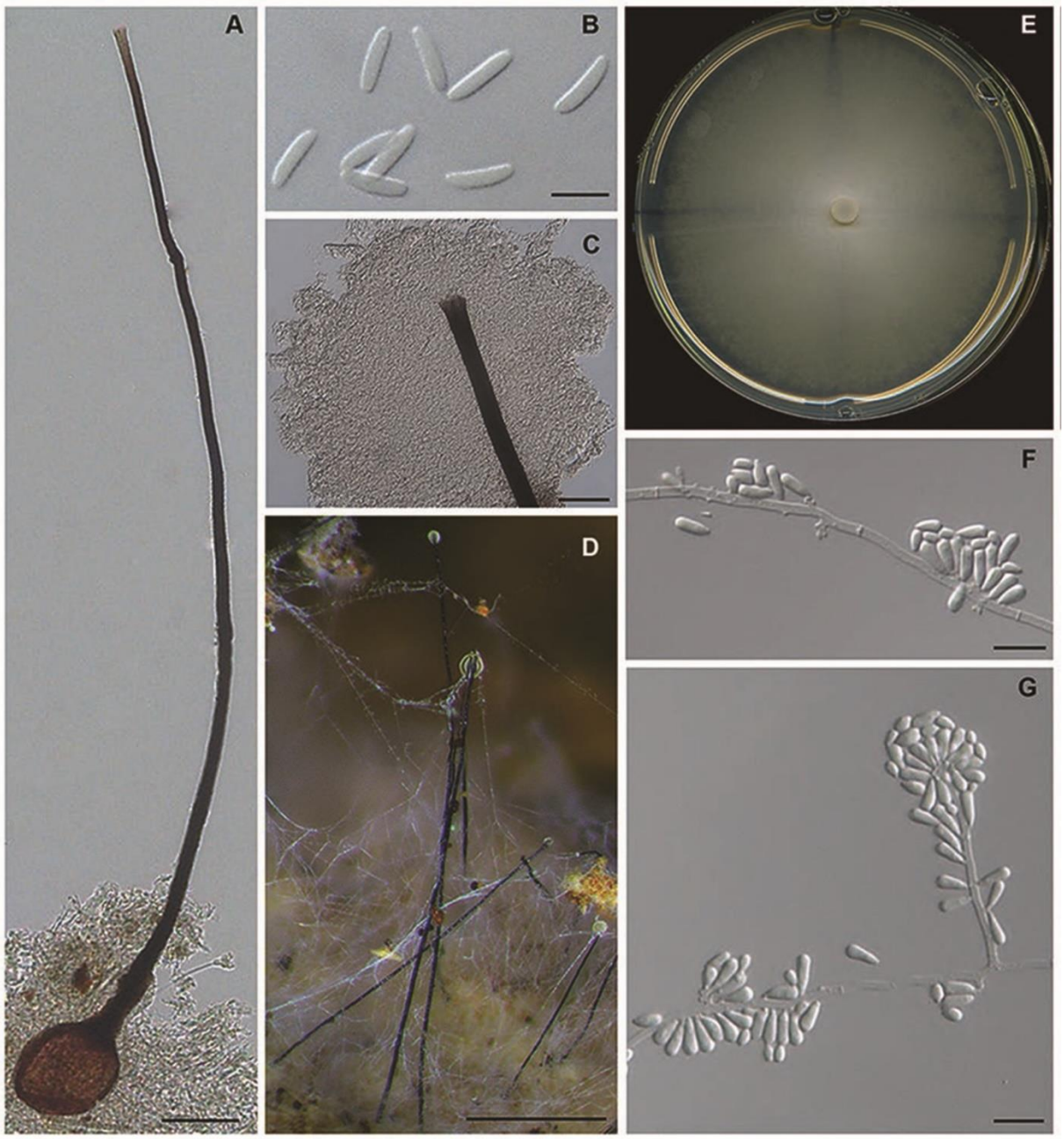


Figure 4. Morphological characteristics of sexual and asexual structures of *Intubia macrotermitinarum* sp. nov. (CMW 46496). A. Long necked ascoma; B. Ascospores; C. Apex without ostiolar hyphae; D. Ascoma on termite fungus comb with sticky drop of ascospores; E. Pure culture on MEA; F. Conidia formation on hyphae; G. Conidiophore with conidia. Bar: D = 500 µm; A = 100 µm; C = 50 µm; F, G = 10 µm; B = 5 µm

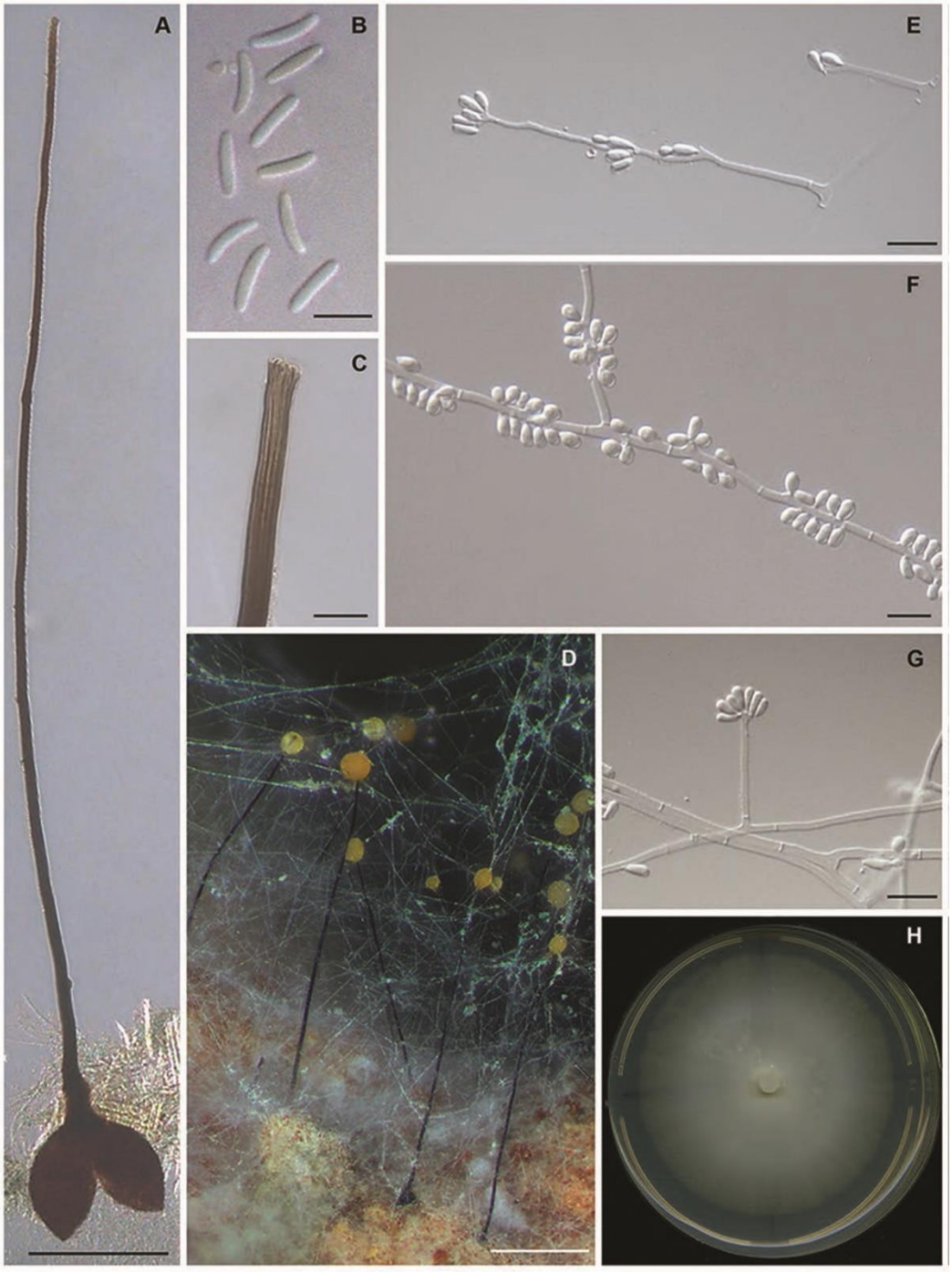
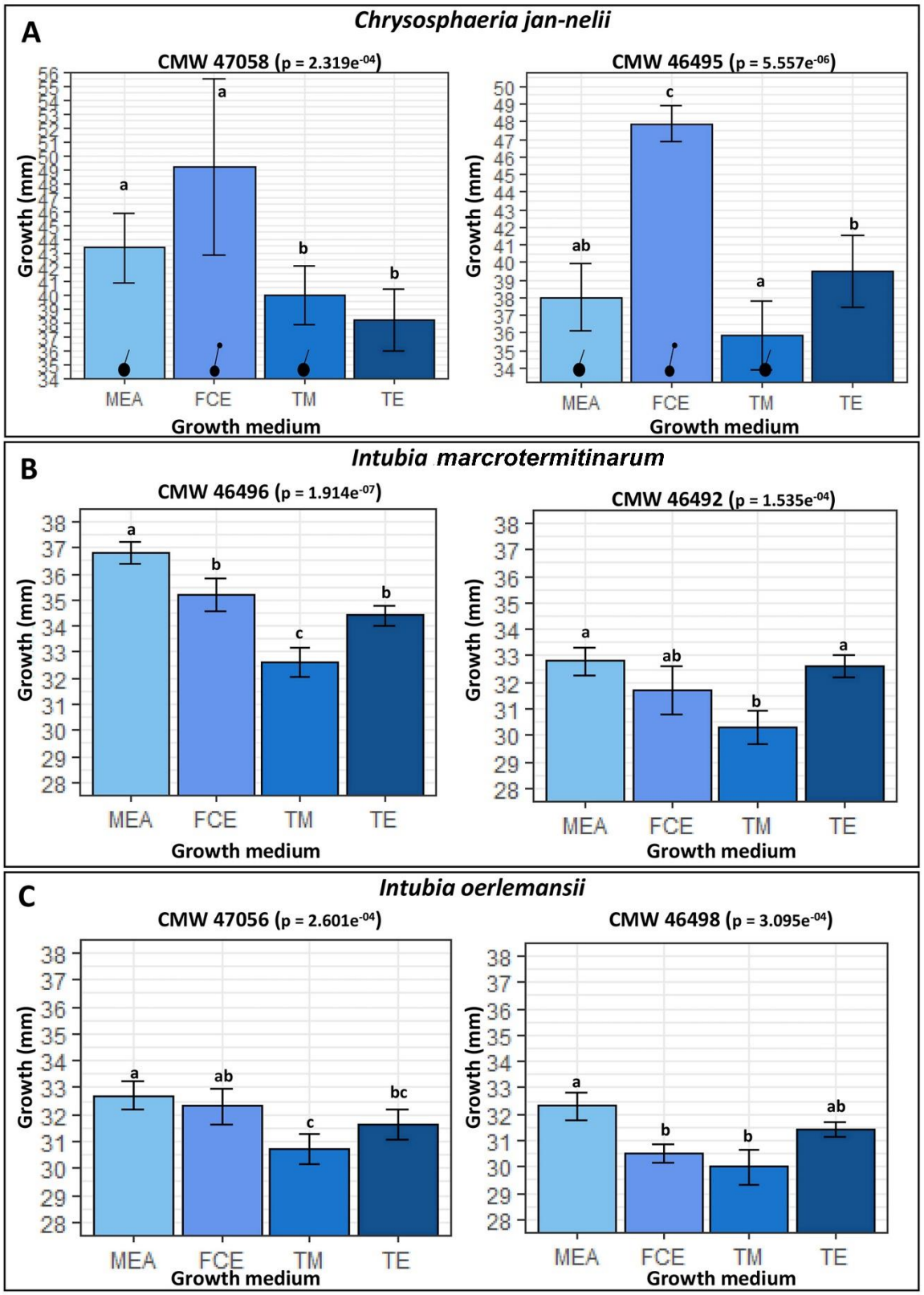


Figure 5. Morphological characteristics of sexual and asexual structures of *Intubia oerlemansii* sp. nov. (CMW 47056). A. Long-necked ascoma; B. Ascospores; C. Apex without osiolar hyphae; D. Ascomata on termite fungus comb with ascospores in slimy droplet; E. and G. Conidia produced on conidiophores; F. Conidia produced on hyphae; H. Pure culture on MEA. Bar: D = 500µm; A = 200 µm; C = 20 µm; E, F, G = 10 µm; B = 5 µm

Figure 6. Bar graphs of the average colony growth on the four different supplemented media types (MEA, FCE, TE, TM) after 8 days of incubation at 25 °C. The data is presented for this day because isolates of *Chrysosphaeria jan-nelii* had completely covered the surface of the FCE media plates and this represented the last day of measurements for the set (set refers to the entire group of five different types of media plates onto which the isolate was plated). *P*-values determined using the Kruskal-Wallis test, significances determined by the Dunn multiple comparisons test, and perithecial development observed on certain media is indicated. A. Results for isolates of *C. jan-nelii*. B. Results for isolates of *I. macrotermitinarum*. C. Results for isolates of *I. oerlemansii*.



Legend

Full perithecia development

Protoperithecia development

p = p-value obtained by Kruskal-Wallis rank sum test
$p \leq 0.05$ indicates significance

a/b/c= significant variation between treatments determined by Dunn's test

References

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A. & Dvorkin, M. (2012). SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology*, 19, 455–477.
DOI: https://doi.org/10.1089/cmb.2012.0021

Bateman, C., Huang, Y-T., Simmons, D.R., Kasson, M.T., Stanley, E.L. & Hulcr, J. (2017). Ambrosia beetle *Premnobius cavipennis* (Scolytinae: Ipini) carries highly divergent ascomycotan ambrosia fungus, *Afroraffaelea ambrosiae* gen. nov. et sp. nov. (Ophiostomatales). *Fungal Ecology*, 25,41-49.
DOI: http://dx.doi.org/10.1016/j.funeco.2016.10.008

Benny, G.L. & Kimbrough, J.W. (1980). A synopsis of the orders and families of Olectomycetes with keys to genera. *Mycotaxon* 12: 1–91.

Blanco-Ulate, B., Rolshausen, P.E. & Cantu, D. (2013). Draft genome sequence of the grapevine dieback fungus *Eutypa lata* UCR-EL1. *Genome Announcements*, 1, e00228–00213.
DOI: https://doi.org/10.1128/genomeA.00228-13

Boetzer, M., Henkel, C.V., Jansen, H.J., Butler. D. & Pirovano, W. (2011). Scaffolding pre-assembled contigs using SSPACE. *Bioinformatics*, 27, 578–579.
DOI: https://doi.org/10.1093/bioinformatics/btq683

Boetzer, M. & Pirovano, W. (2012). Toward almost closed genomes with GapFiller. *Genome Biology*. 13, R56.
DOI: https://doi.org/10.1186/gb-2012-13-6-r56

Bolger, A.M., Lohse, M. & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, 30, 2114–2120.
DOI: https://doi.org/10.1093/bioinformatics/btu170

Cantarel, B.L., Korf, I., Robb, S.M., Parra, G., Ross, Eet al. (2008). MAKER: An easy-to-use annotation pipeline designed for emerging model organism genomes. *Genome Research*, 18, 188–196.
DOI: https://doi.org/10.1101/gr.6743907

Capella-Gutiérrez, S., Silla-Martínez, J.M. & Gabaldón, T. (2009). trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, 25, 1972–1973.
DOI: https://doi.org/10.1093/bioinformatics/btp348

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, 17, 540-552.
DOI: https://doi.org/10.1093/oxfordjournals.molbev.a026334

Chang, R., Duong, T.A., Taerum, S.J., Wingfield, M.J., Zhou, X. & De Beer, Z.W. (2020). Ophiostomatoid fungi associated with mites phoretic on bark beetles in Qinghai, China. *IMA Fungus*, 11, 15.
DOI: https://doi.org/10.1186/s43008-020-00037-9

D'Alessandro, E., Giosa, D., Huang, L., Zhang, J., Gao, W., et al. (2016). Draft genome sequence of the dimorphic fungus *Sporothrix pallida*, a nonpathogenic species belonging to *Sporothrix* a genus containing agents of human and feline sporotrichosis. *Genome Announcements*, 4, e00184–00116.
DOI: https://doi.org/10.1128/genomeA.00184-16

De Beer, Z.W. & Wingfield, M.J. (2013). Emerging lineages in the Ophiostomatales. In: Seifert, K.A., De Beer, Z.W. & Wingfield, M.J. (Eds) *The Ophiostomatoid Fungi: Expanding Frontiers*, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, pp. 21-46.

De Beer, Z.W., Seifert, K.A. & Wingfield, M.J. (2013a). The Ophiostomatoid fungi: their dual position in the Sordariomycetes. In: Seifert, K.A., De Beer, Z.W. & Wingfield, M.J. (Eds.),

The Ophiostomatoid Fungi: Expanding Frontiers, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, pp. 12, 1-19.

De Beer, Z.W., Seifert, K.A. & Wingfield, M.J. (2013b). A nomenclator for Ophiostomatoid genera and species in the *Ophiostomatales* and *Microascales*. In: Seifert, K.A., De Beer, Z.W. & Wingfield, M.J. (Eds.), *The Ophiostomatoid Fungi: Expanding Frontiers*, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, pp. 245-322.

De Beer, Z.W., Duong, T.A., Barnes, I., Wingfield, B.D. & Wingfield, M.J. (2014). Redefining *Ceratocystis* and allied genera. *Studies in Mycology*, 79, 187-219. https://doi.org/10.1016/j.simyco.2014.10.001

De Beer, Z.W., Duong, T.A. & Wingfield, M.J. (2016a). The divorce of *Sporothrix* and *Ophiostoma*: Solution to a problematic relationship. *Studies in Mycology*, 83, 165–191. DOI: https://doi.org/10.1016/j.simyco.2016.07.001

De Beer, Z.W., Marincowitz, S., Duong, T.A., Kim, J.J., Rodrigues, A. & Wingfield, M.J. (2016b). *Hawksworthiomyces* gen. nov. (Ophiostomatales), illustrates the urgency for a decision on how to name novel taxa known only from environmental nucleic acid sequences (ENAS). *Fungal Biology*, 120, 1323–1340. DOI: https://doi.org/10.1016/j.funbio.2016.07.004

De Lima Barros, M.B., De Oliveira Schubach, A., Do Valle, A.C.F., Galhardo, M.C.G., Conceição-Silva, F., et al. (2004). Cat-transmitted sporotrichosis epidemic in Rio de Janeiro, Brazil: Description of a series of cases. *Clinical Infectious Diseases*, 38, 529–535. DOI: https://doi.org/10.1086/381200

De Meyer, E.M., De Beer, Z.W., Summerbell, R.C., Moharram, A., De Hoog, G.S., et al. (2008). Taxonomy and phylogeny of new wood-and soil-inhabiting *Sporothrix* species in the *Ophiostoma stenoceras-Sporothrix schenckii* complex. *Mycologia*, 100, 647–661. DOI: https://doi.org/10.3852/07-157R

Dean, R.A., Talbot, N.J., Ebbole, D.J., Farman, M.L., Mitchell, T.K., et al. (2005). The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature*, 434, 980-986. DOI: https://doi.org/10.1038/nature03449

DiGuistini, S., Wang, Y., Liao, N.Y., Taylor, G., Tanguay, P., et al. (2011). Genome and transcriptome analyses of the mountain pine beetle-fungal symbiont *Grosmannia clavigera*, a lodgepole pine pathogen. *Proceedings of the National Academy of Sciences*, 108, 2504–2509. DOI: https://doi.org/10.1073/pnas.1011289108

Dunn, O.J. (1964) Multiple comparisons using rank sums. *Technometrics*, 6, 241–252.

Duong, T.A., De Beer, Z.W., Wingfield, B.D., & Wingfield, M.J. (2012). Phylogeny and taxonomy of species in the *Grosmannia serpens* complex. *Mycologia*, 104, 715–732. DOI: https://doi.org/10.3852/11-109

Duong, T.A., De Beer, Z.W., Winfield, B.D. & Wingfield, M.J. (2013). Characterization of the mating-type genes in *Leptographium procerum* and *Leptographium profanum*. *Fungal Biology*, 117, 411–421. DOI: https://doi.org/10.1016/j.funbio.2013.04.005

Edler, D., Klein, J., Antonelli, A. & Silvestro, D. (2019). raxmlGUI 2.0 beta: A graphical interface and toolkit for phylogenetic analyses using RAxML. *BioRxiv*, 800912. DOI: https://doi.org/10.1101/800912

Eickwort, G.C. (1990). Associations of mites with social insects. *Annual Review of Entomology*, 35, 469-488. DOI: https://doi.org/10.1146/annurev.en.35.010190.002345

Emms, D. & Kelly, S. (2018). STAG: Species tree inference from all genes. *BioRxiv*, 267914. DOI: https://doi.org/10.1101/267914

Flutre, T., Duprat, E., Feuillet, C. & Quesneville, H. (2011). Considering transposable element diversification in *de novo* annotation approaches. *PLoS ONE*, 6, e16526. DOI: https://doi.org/10.1371/journal.pone.0016526

Forgetta, V., Leveque, G., Dias, J., Grove, D., Lyons, R., et al. (2013). Sequencing of the Dutch elm disease fungus genome using the Roche/454 GS-FLX Titanium System in a comparison of multiple genomics core facilities. *Journal of Biomolecular Techniques*, 24, 39–49. DOI: https://doi.org/10.7171/jbt.12-2401-005

Galagan, J.E., Calvo, S.E., Borkovich, K.A., Selker, E.U., Read, N.D., et al. (2003). The genome sequence of the filamentous fungus *Neurospora crassa*. *Nature*, 422, 859–868. DOI: https://doi.org/10.1038/nature01554

Gardes, M. & Bruns, T.D. (1993). ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. *Molecular Ecology*, 2, 113-118. DOI: https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Geiser, D.M., Al-Hatmi, A., Aoki, T., Arie, T., Balmas, V., Barnes, I., et al. (2020) Phylogenomic analysis of a 55.1 kb 19-gene dataset resolves a monophyletic *Fusarium* that includes the *Fusarium solani* species complex. *Phytopathology*. DOI: https://doi.org/10.1094/PHYTO-08-20-0330-LE

Glass, N.L. & Donaldson, G.C. (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology*, 61, 1323-1330.

Haridas, S., Wang, Y., Lim, L., Massoumi-Alamouti, S., Jackman, S., et al. (2013). The genome and transcriptome of the pine saprophyte *Ophiostoma piceae*, and a comparison with the bark beetle-associated pine pathogen *Grosmannia clavigera*. *BMC Genomics*, 14, 373. DOI: https://doi.org/10.1186/1471-2164-14-373

Harrington, T.C., Aghayeva, D.N. & Fraedrich, S. (2010). New combinations in *Raffaelea*, *Ambrosiella*, and *Hyalorhinocladiella*, and four new species from the redbay ambrosia beetle, *Xyleborus glabratus*. *Mycotaxon*, 111, 337-361. DOI: https://doi.org/10.5248/111.337

Hausner, G., Reid, J. & Klassen, G.R. (1993). On the phylogeny of *Ophiostoma*, *Ceratocystis* s.s., and *Microascus*, and the relationships within *Ophiostoma* based on partial ribosomal DNA sequences. *Canadian Journal of Botany*, 71, 1249-1265. DOI: https://doi.org/10.1139/b93-148

Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q., Vinh, L.S. (2017). UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, 35, 518-522. DOI: https://doi.org/10.1093/molbev/msx281

Hawksworth, D.L., Crous, P.W., Redhead, S.A., Reynolds, D.R., Samson, R.A., et al. (2011). The Amsterdam declaration on fungal nomenclature. *IMA Fungus*, 2, 105–111. DOI: https://doi.org/10.5598/imafungus.2011.02.01.14

Hofstetter, R.W., Moser, J. & Blomquist, S. (2013). Mites associated with bark beetles and their hyperphoretic Ophiostomatoid fungi. In: Seifert, K.A., De Beer, Z.W. & Wingfield, M.J. (Eds.) *The Ophiostomatoid Fungi: Expanding Frontiers*, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, pp. 165-176.

Huang, L., Gao, W., Giosa, D., Criseo, G., Zhang, J., et al. (2016). Whole-genome sequencing and *in silico* analysis of two strains of *Sporothrix globosa*. *Genome Biology and Evolution*, 8, 3292–3296. DOI: https://doi.org/10.1093/gbe/evw230

Jacobs, K., Wingfield, M.J. & Wingfield, B.D. (2001). Phylogenetic relationships in *Leptographium* based on morphological and molecular characters. *Canadian Journal of Botany* ,79, 719–732.

DOI: https://doi.org/10.1139/b01-041

Jeon, J., Kim, K.T., Song, H., Lee, G.W., Cheong, K., et al. (2017). Draft genome sequence of the fungus associated with oak wilt mortality in South Korea *Raffaelea quercus-mongolicae* KACC44405. *Microbiology Resource Announcements*, 5, e00797–00717.
DOI: https://doi.org/10.1128/genomeA.00797-17

Katoh, K. & Standley, D.M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, 30, 772-780.
DOI: https://doi.org/10.1093/molbev/mst010

Khoshraftar, S., Hung, S., Khan, S., Gong, Y., Tyagi, V., et al. (2013). Sequencing and annotation of the *Ophiostoma ulmi* genome. *BMC Genomics*, 14, 162.
DOI: https://doi.org/10.1186/1471-2164-14-162

Kirisits, T. (2013). Dutch elm disease and other *Ophiostoma* diseases. In: Gonthier, P. & Nicolotti, G. (Eds.), *Infectious Forest Diseases*. CABI Publishing. pp. 256–282.

Kruskal, W.H. & Wallis, W.A. (1952). Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association*, 47, 583–621.

Kück, P. & Meusemann, K. (2010). FASconCAT: Convenient handling of data matrices. *Molecular Phylogenetics and Evolution*, 56, 1115-1118.
DOI: https://doi.org/10.1016/j.ympev.2010.04.024

Lah, L., Löber, U., Hsiang, T. & Hartmann, S. (2017). A genomic comparison of putative pathogenicity-related gene families in five members of the Ophiostomatales with different lifestyles. *Fungal Biology*, 121, 234–252.
DOI: https://doi.org/10.1016/j.funbio.2016.12.002

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T. & Calcott, B. (2017). PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, 34, 772–773.
DOI: https://doi.org/10.1093/molbev/msw260

Liu, F., Chen, S., Ferreira, M.A., Chang, R., Sayari, M., et al. (2019). Draft genome sequences of five *Calonectria* species from *Eucalyptus* plantations in China, *Celoporthe dispersa*, *Sporothrix phasma* and *Alectoria sarmentosa*. *IMA Fungus*, 10, 1–13.
DOI: https://doi.org/10.1186/s43008-019-0023-5

Lomsadze, A., Ter-Hovhannisyan, V., Chernoff, Y.O. & Borodovsky, M. (2005). Gene identification in novel eukaryotic genomes by self-training algorithm. *Nucleic Acids Research*, 33, 6494–6506.
DOI: https://doi.org/10.1093/nar/gki937

Malloch DW, Blackwell M. (1993). Dispersal biology of the Ophiostomatoid fungi. In: Wingfield, M.J., Seifert, K.A. & Webber, J. (Eds.) Ceratocystis *and* Ophiostoma*: Taxonomy, Ecology and Pathogenicity.* St. Paul, Minnesota: APS Press. pp. 195–206.

Massoumi-Alamouti, S., Tsui, C.K. & Breuil, C. (2009). Multigene phylogeny of filamentous ambrosia fungi associated with ambrosia and bark beetles. *Mycological Research*, 113, 822-835.
DOI: https://doi.org/10.1016/j.mycres.2009.03.003

Masuya, H., Manabe, R-I., Ohkuma, M. & Endoh, R. (2016). Draft genome sequence of *Raffaelea quercivora* JCM 11526, a Japanese oak wilt pathogen associated with the platypodid beetle, *Platypus quercivorus*. *Microbiology Resource Announcements*, 4, e00755–00716.
DOI: https://doi.org/ 10.1128/genomeA.00755-16

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., et al. (2020). IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, 37, 1530-1534.
DOI: https://doi.org/10.1093/molbev/msaa015

Mirarab, S., Reaz, R., Bayzid, M.S., Zimmermann, T., Swenson, M.S. & Warnow, T. (2014). ASTRAL: Genome-scale coalescent-based species tree estimation. *Bioinformatics*, 30, i541–i548. DOI: https://doi.org/10.1093/bioinformatics/btu462

Morales-Cruz, A., Amrine, K.C.H., Blanco-Ulate, B., Lawrence, D.P., Travadon, R., et al. (2015). Distinctive expansion of gene families associated with plant cell wall degradation, secondary metabolism, and nutrient uptake in the genomes of grapevine trunk pathogens. *BMC Genomics*, 16, 469. DOI: https://doi.org/10.1186/s12864-015-1624-z

Moriya, S., Inoue, T., Ohkuma, M., Yaovapa, T., Johjima, T., et al. (2005). Fungal community analysis of fungus gardens in termite nests. *Microbes and Environments*, 20, 243–252. DOI: https://doi.org/10.1264/jsme2.20.243

O'Donnell, K & Cigelnik, E. (1997). Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are non-orthologous. *Molecular Phylogenetics and Evolution*, 7, 103-116. DOI: http://dx.doi.org/10.1006/mpev.1996.0376

Otani, S., Challinor, V.L., Kreuzenbeck, N.B., Kildgaard, S., Christensen, S.K., et al. (2019). Disease-free monoculture farming by fungus-growing termites. *Scientific Reports*, 9, 8819. DOI: https://doi.org/10.1038/s41598-019-45364-z

Quesneville, H., Bergman, C.M., Andrieu, O., Autard, D., Nouaud, D., et al. (2005). Combined evidence annotation of transposable elements in genome sequences. *PLoS Computational Biology*, 1, e22. DOI: https://doi.org/10.1371/journal.pcbi.0010022

Roets, F., Wingfield, B.D., De Beer, Z.W., Wingfield, M.J. & Dreyer, L.L. (2010). Two new *Ophiostoma* species from *Protea caffra* in Zambia. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, 24, 18-28. DOI: https://doi.org/10.3767/003158510X490392

Ronquist, F. & Huelsenbeck, J.P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 17, 1572-1574. DOI: https://doi.org/10.1093/bioinformatics/btg180

Salichos, L. & Rokas, A. (2013). Inferring ancient divergences requires genes with strong phylogenetic signals. *Nature*, 497, 327–331. DOI: https://doi.org/10.1038/nature12130

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., et al. (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proceedings of the National Academy of Sciences*, 109, 6241-6246. DOI: https://doi.org/10.1073/pnas.1117018109

Seppey, M., Manni, M. & Zdobnov, E.M. (2019). BUSCO: Assessing genome assembly and annotation completeness. *Gene Prediction*, 3, 227–245. DOI: https://doi.org/10.1007/978-1-4939-9173-0_14

Shen, X.X., Hittinger, C.T. & Rokas, A. (2017). Contentious relationships in phylogenomic studies can be driven by a handful of genes. *Nature Ecology & Evolution*, 1, 0126. DOI: https://doi.org/10.1038/s41559-017-0126

Silvestro, D. & Michalak, I. (2012). raxmlGUI: A graphical front-end for RAxML. *Organism Diversity and Evolution*, 12, 335-337. DOI: https://doi.org/10.1007/s13127-011-0056-0

Six, D.L. (2012). Ecological and evolutionary determinants of bark beetle—fungus symbioses. *Insects*, 3, 339-366. DOI: https://doi.org/10.3390/insects3010339

Spatafora, J.W. & Blackwell, M. (1994). The polyphyletic origins of Ophiostomatoid fungi. *Mycological Research*, 98, 1-9.
DOI: https://doi.org/10.1016/S0953-7562(09)80327-4

Stamatakis, A. (2006). RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22, 2688–2690.
DOI: https://doi.org/10.1093/bioinformatics/btl446

Stanke, M., Keller, O., Gunduz, I., Hayes, A., Waack, S. & Morgenstern, B. (2006). AUGUSTUS: *Ab initio* prediction of alternative transcripts. *Nucleic Acids Research*, 34, W435–W439.
DOI: https://doi.org/10.1093/nar/gkl200

Stielow, J.B., Levesque, C.A., Seifert, K.A., Meyer, W., Iriny, L., et al. (2015). One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, 35: 242.
DOI: https://doi.org/10.3767/003158515X689135

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725–2729.
DOI: https://doi.org/10.1093/molbev/mst197

Teixeira, M.M., De Almeida, L.G.P., Kubitschek-Barreira, P., Alves, F.L., Kioshima, E.S., et al. (2014). Comparative genomics of the major fungal agents of human and animal Sporotrichosis: *Sporothrix schenckii* and *Sporothrix brasiliensis*. *BMC Genomics*, 15, 943.
DOI: https://doi.org/10.1186/1471-2164-15-943

Ter-Hovhannisyan, V., Lomsadze, A., Chernoff, Y.O. & Borodovsky, M. (2008). Gene prediction in novel fungal genomes using an ab initio algorithm with unsupervised training. *Genome Research*, 18, 1979–1990.
DOI: https://doi.org/10.1101/gr.081612.108

Van Der Linde, J.A., Six, D.L., De Beer, W.Z., Wingfield, M.J. & Roux, J. (2016). Novel ophiostomatalean fungi from galleries of *Cyrtogenius africus* (Scolytinae) infesting dying *Euphorbia ingens*. *Antonie van Leeuwenhoek*, 109, 589-601.
DOI: https://doi.org/ 10.1007/s10482-016-0661-1

Van Der Nest, M.A., Bihon, W., De Vos, L., Naidoo, K., Roodt, D., et al. (2014). Draft genome sequences of D*iplodia sapinea*, *Ceratocystis manginecans*, and *Ceratocystis moniliformis*. *IMA Fungus*, 5, 135–140.
DOI: https://doi.org/10.5598/imafungus.2014.05.01.13

Vanderpool, D., Bracewell, R.R. & McCutcheon, J.P. (2018). Know your farmer: Ancient origins and multiple independent domestications of ambrosia beetle fungal cultivars. *Molecular Ecology*, 27, 2077-2094.
DOI: https://doi.org/10.1111/mec.14394

Vilgalys, R. & Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology*, 172, 4238-4246.
DOI: https://doi.org/10.1128/jb.172.8.4238-4246.1990

Visser, A.A., Kooij, P.W., Debets, A.J., Kuyper, T.W. & Aanen, D.K. (2011). *Pseudoxylaria* as stowaway of the fungus-growing termite nest: Interaction asymmetry between *Pseudoxylaria*, *Termitomyces* and free-living relatives. *Fungal Ecology*, 4, 322–332.
DOI: https://doi.org/10.1016/j.funeco.2011.05.003

Visser, A.A., Ros, V.I.D., De Beer, Z.W., Debets, A.J.M., Hartog, E., et al. (2009). Levels of specificity of *Xylaria* species associated with fungus-growing termites: A phylogenetic approach. *Molecular Ecology*, 18, 553–567.
DOI: https://doi.org/10.1111/j.1365-294X.2008.04036.x

Wang, C., Powell, J.E. & O'Connor, B.M. (2002). Mites and nematodes associated with three subterranean termite species (Isoptera: Rhinotermitidae). *Florida Entomologist*, 85, 499-506. DOI: https://doi.org/10.1653/0015-4040(2002)085[0499:MANAWT]2.0.CO;2

White T.J., Bruns T., Lee S., Taylor J.W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sinsky, J.J., White, T.J. (Eds.), *PCR Protocols: A Guide to the Methods and Applications*. New York: Academic Press, New York, pp. 315–322.

Wingfield, B.D., Ades, P.K., Al-Naemi, F.A., Beirn, L.A., Bihon, W., et al. (2015a). Draft genome sequences of *Chrysoporthe austroafricana, Diplodia scrobiculata, Fusarium nygamai, Leptographium lundbergii, Limonomyces culmigenus, Stagonosporopsis tanaceti,* and *Thielaviopsis punctulata*. *IMA Fungus*, 6, 233–248. DOI: https://doi.org/10.5598/imafungus.2015.06.01.15

Wingfield, B.D., Barnes, I., De Beer, Z.W., De Vos, L., Duong, T.A., et al. (2015b.). Draft genome sequences of *Ceratocystis eucalypticola, Chrysoporthe cubensis, C. deuterocubensis, Davidsoniella virescens, Fusarium temperatum, Graphilbum fragrans, Penicillium nordicum,* and *Thielaviopsis musarum*. *IMA Fungus*, 6, 493–506. DOI: https://doi.org/10.5598/imafungus.2015.06.02.13

Wingfield, B.D., Ambler, J.M., Coetzee, M.P.A., De Beer, Z.W., Duong, T.A., et al.(2016a). Draft genome sequences of *Armillaria fuscipes, Ceratocystiopsis minuta, Ceratocystis adiposa, Endoconidiophora laricicola, E. polonica* and *Penicillium freii* DAOMC 242723. *IMA Fungus*, 7, 217–227. DOI: https://doi.org/10.5598/imafungus.2016.07.01.11

Wingfield, B.D., Duong, T.A., Hammerbacher, A., Van Der Nest, M.A., Wilson, A., et al. (2016b). Draft genome sequences for *Ceratocystis fagacearum, C. harringtonii, Grosmannia penicillata,* and *Huntiella bhutanensis*. *IMA Fungus*, 7, 317–323. DOI: https://doi.org/10.5598/imafungus.2016.07.02.11

Wingfield, B.D., Berger, D.K., Steenkamp, E.T., Lim, H.J., Duong, T.A., et al. (2017). Draft genome of *Cercospora zeina, Fusarium pininemorale, Hawksworthiomyces lignivorus, Huntiella decipiens* and *Ophiostoma ips*. *IMA Fungus*, 8, 385–396. DOI: https://doi.org/10.5598/imafungus.2017.08.02.10

Wingfield, B.D., Liu, M., Nguyen, H.D., Lane, F.A., Morgan, S.W., et al. (2018). Nine draft genome sequences of *Claviceps purpurea* s. lat., including *C. arundinis, C. humidiphila*, and *C. cf. spartinae*, pseudomolecules for the pitch canker pathogen *Fusarium circinatum*, draft genome of *Davidsoniella eucalypti*, *Grosmannia galeiformis*, *Quambalaria eucalypti*, and *Teratosphaeria destructans*. *IMA Fungus*, 9, 401-418. DOI: https://doi.org/10.5598/imafungus.2018.09.02.10

Yin, M., Duong, T.A., Wingfield, M.J., Zhou, X. & De Beer, Z.W. (2015). Taxonomy and phylogeny of the *Leptographium procerum* complex, including *Leptographium sinense* sp. nov. and *Leptographium longiconidiophorum* sp. nov. *Antonie van Leeuwenhoek*, 107, 547–563. DOI: https://doi.org/10.1007/s10482-014-0351-9

Zipfel, R.D., De Beer, Z.W., Jacobs, K., Wingfield, B.D. & Wingfield, M.J. (2006). Multi-gene phylogenies define *Ceratocystiopsis* and *Grosmannia* distinct from *Ophiostoma*. *Studies in Mycology*, 55, 75-97.

DOI: https://doi.org/10.3114/sim.55.1.75

Supplementary Table1. All Fungal isolates obtained and sequenced in this study

Species	Isolate number			Geographical Origin	Host	GenBank accession numbers		
	CMW	**CBS**	**PREM**			**ITS**	**LSU**	***βT***
Lineage A: *Chrysosphaeria jan-nelii*	48039			Amsterdam farm, Mookgophong, Limpopo, SA	Fungus comb from termite mounds	MT637036	MT637009	
	47057	141566	63087	Amsterdam farm, Mookgophong, Limpopo, SA	Fungus comb from termite mounds	MT637037	MT637008	
	47058[a]	**141570**	**63088**	Amsterdam farm, Mookgophong, Limpopo, SA	Fungus comb from termite mounds	MT637038	MT637006	MT649128
	46495[a]	141571	63089	Amsterdam farm, Mookgophong, Limpopo, SA	Fungus comb from termite mounds	MT637042	MT637007	
Lineage B1: *Intubia macrotermitinarum*	47040			University of Pretoria, Experimental farm, Pretoria, SA	Fungus comb from termite mounds	MT637014		MT649139
	47041			University of Pretoria, Experimental farm, Pretoria, SA	Fungus comb from termite mounds	MT637015		MT649140
	47042		63092	University of Pretoria, Experimental farm, Pretoria, SA	Fungus comb from termite mounds	MT637016		MT649141
	47043			University of Pretoria, Experimental farm, Pretoria, SA	Fungus comb from termite mounds	MT637017		MT649142
	47044			University of Pretoria, Experimental farm, Pretoria, SA	Fungus comb from termite mounds	MT637019	MT63695	MT649143
	47047			University of Pretoria, Experimental farm, Pretoria, SA	Fungus comb from termite mounds	MT637023		MT649146
	46496[a]	**141560**	**63090**	PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637025	MT636994	MT649132
	47049			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637026		MT649148
	47050		93093	PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637027		MT649149
	47051			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637028	MT645590	MT649150
	46492[a]	141561	63091	University of Pretoria, Experimental farm, Pretoria, SA	Fungus comb from termite mounds	MT637030		MT649129
	47053			University of Pretoria, Experimental farm, Pretoria, SA	Fungus comb from termite mounds	MT637031	MT645591	MT649152
	47060			University of Pretoria, Experimental farm, Pretoria, SA	Fungus comb from termite mounds	MT637041	MT636992	MT649156
	47063			University of Pretoria, Experimental farm, Pretoria, SA	Fungus comb from termite mounds	MT637045		MT649159
	47064			University of Pretoria, Experimental farm, Pretoria, SA	Fungus comb from termite mounds	MT637046		MT649160
	47065			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637047	MT645588	MT649161
	47066			Amsterdam farm, Mookgophong, Limpopo, SA	Fungus comb from termite mounds	MT637048	MT636993	MT649162

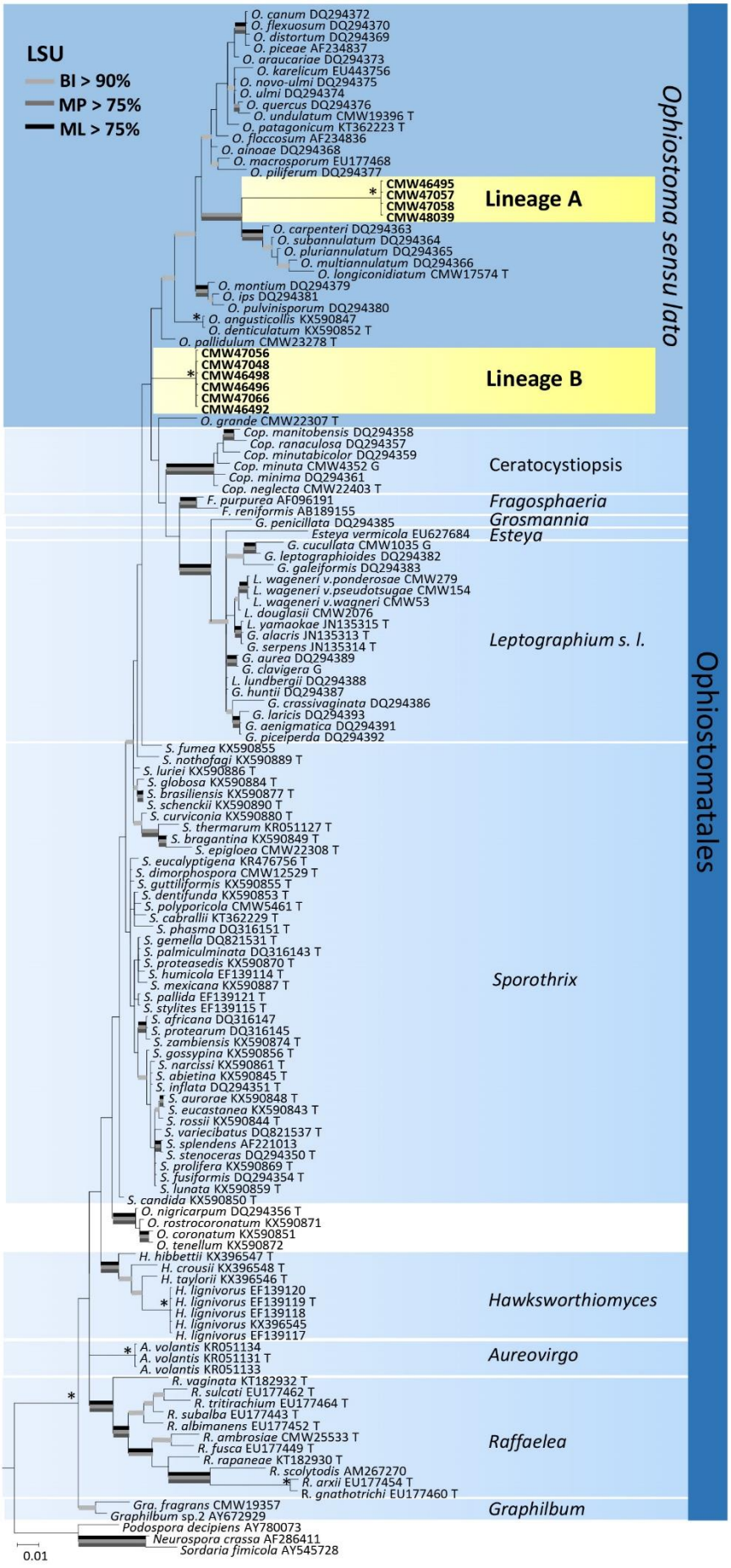
Species	Isolate number			Geographical Origin	Host	GenBank accession numbers		
	CMW	**CBS**	**PREM**			**ITS**	**LSU**	***βT***
	47067	141562		Amsterdam farm, Mookgophong, Limpopo, SA	Fungus comb from termite mounds	MT637049	MT645589	MT649163
	47068			Amsterdam farm, Mookgophong, Limpopo, SA	Fungus comb from termite mounds	MT637050		MT649164
	47069			University of Pretoria, Experimental farm, Pretoria, SA	Fungus comb from termite mounds	MT637060	MT636997	MT649165
	46494			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637061		MT649121
	47070			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637062		MT649166
	47072			University of Pretoria, Experimental farm, Pretoria, SA	Fungus comb from termite mounds	MT067064		MT649168
	47074			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637066	MT636991	MT649170
	48941				Fungus comb from termite mounds	MT835247		PENDING
Lineage B2: *Intubia oerlemansii*	47036			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637010		MT649135
	47037			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637011		MT649136
	47038			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637012		MT649137
	47039			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637013	MT636999	MT649138
	47045			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637020	MT636998	MT649144
	46497			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637021		MT649133
	47046			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637022		MT649145
	47048	**141564**	**63094**	PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637024		MT649147
	47052			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637029	MT637002	MT649151
	47054			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637032		MT649153
	47055		63095	PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637033	MT637000	MT649154
	47056[a]	141565	63096	PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637034	MT637001	MT708983
	46493		63097	PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637035		MT649130

Species	Isolate number			Geographical Origin	Host	GenBank accession numbers		
	CMW	**CBS**	**PREM**			**ITS**	**LSU**	***βT***
	47059			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637039		MT649155
	46498[a]	141563	63098	PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637040	MT940646	MT649134
	47061			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637043		MT649157
	47062			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637044	MT636996	MT649158
	47071			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637063		MT649167
	47073			PPRI, Rietondale, Pretoria, SA	Fungus comb from termite mounds	MT637065		MT649169
	48942				Fungus comb from termite mounds	MT835248		PENDING

[a]Isolates used in growth studies

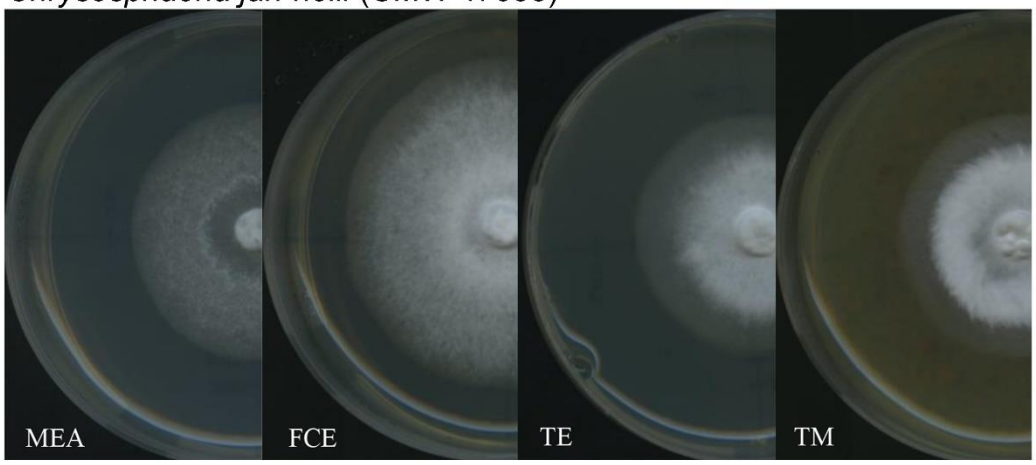
Bold face = ex-type isolates

Supplementary Figure 1. Bayesian inference tree derived from analysis of LSU data including all major groups in the Ophiostomatales. Bold light grey bands indicate posterior probabilities above 0.95, medium grey bands indicate MP bootstrap values above 75%, and black bands indicate ML bootstrap values above 75%. Asteriks (*) indicates a node with full support from BI, ML, and MP analyses. T = ex-type isolates

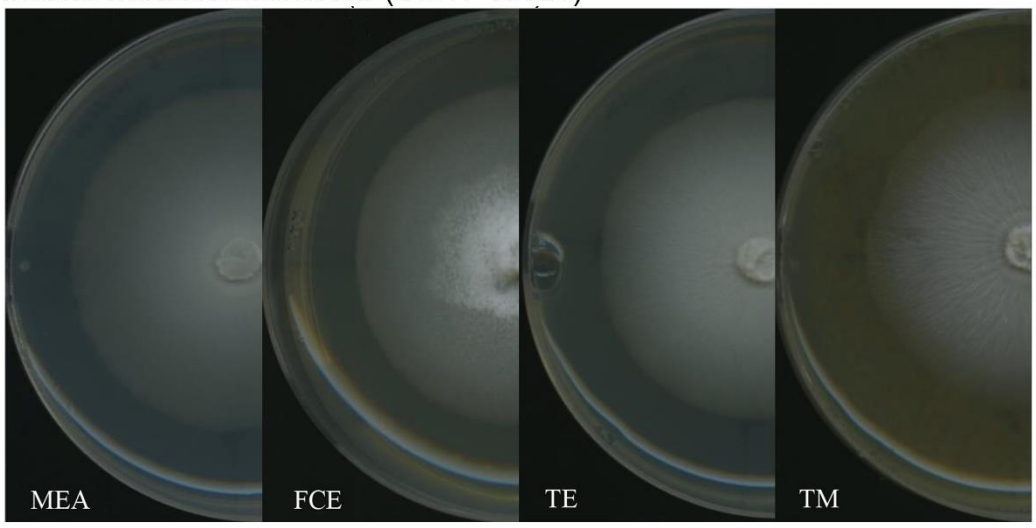


Supplementary Figure 4. Representatives of the new species grown on MEA and different *Termitomyces* enriched media plates.

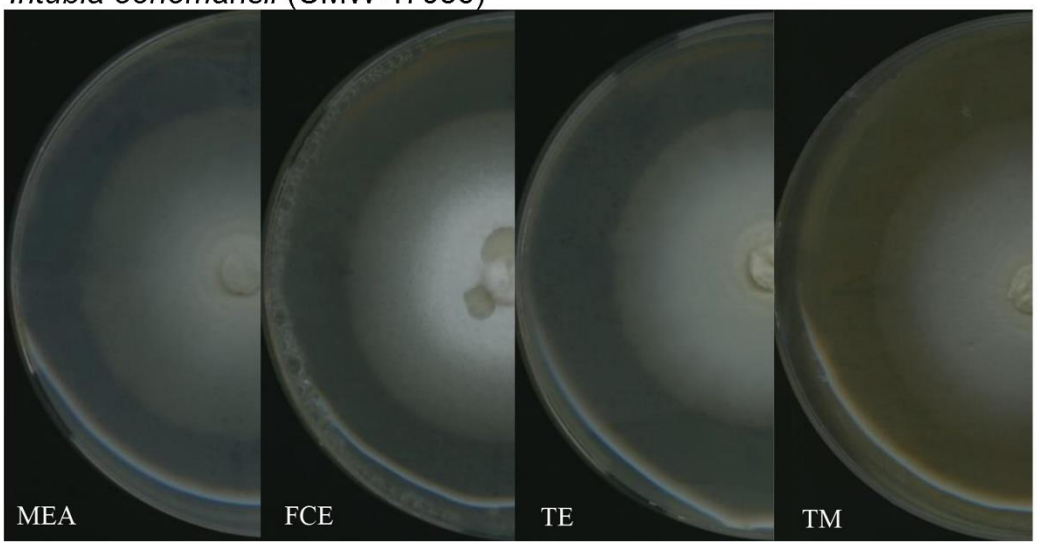
Chrysosphaeria jan-nelii (CMW 47058)



Intubia macrotermitinarum (CMW 46496)



Intubia oerlemansii (CMW 47056)



CHAPTER 5

Convergent patterns of genome reduction and gene loss in arthropod mutualistic Sordariomycete fungi

Convergent patterns of genome reduction and gene loss in arthropod mutualistic Sordariomycete fungi

1. Abstract

Many animals, plants and microbes share complex beneficial relationships known as mutualisms. Of the approximately 30 known Sordariomycete orders, at least three (Ophiostomatales, Microascales and Hypocreales) include species that have evolved sophisticated mutualism with arthropod vectors, but very little is known regarding the molecular characteristics associated with this intriguing lifestyle. In this study, we determined the genomic patterns associated with arthropod mutualisms in members of the Sordariomycetes. By comparing various genomic characters for over 100 arthropod-mutualistic and non-mutualistic fungal species, the genomes of arthropod mutualistic fungi were shown to be smaller than those of their non-mutualistic relatives. Evidence also emerged that this genome size reduction is related to a common trend of lineage-specific loss of pathogenicity-related genes. Overall, the study suggests that long-standing mutualism with arthropod partners has led to genomic convergence of arthropod- mutualistic fungi.

2. Introduction

The term symbiosis was introduced in the late 19th century to describe the communal life of two unlike organisms (Frank 1877, De Bary 1879, Martin 2012). However, a contemporary application of this term accommodates substantially more than simply the co-existence of two organisms. Complex relationships including mutualism, antagonism and commensalism can all collectively be considered using the single term symbiosis (De Bary 1879, Martin 2012).

One of the most sophisticated symbioses known in nature is that of insect fungiculture. Aside from the agricultural practices of humans, all other known forms of agriculture in animals are those involving insects (Mueller et al. 2005). Due to their cultivation habits and obligate association with mutualistic fungi, three groups in the Kingdom Insecta are considered true fungus-farmers. These are macrotermitinae termites, attine ants and ambrosia beetles (Farrell et al. 2001, Mueller et al. 2005).

Ambrosia beetles primarily associate with Ascomycete fungi residing in the Class Sordariomycota (Hulcr & Stelinski 2017). However, this diverse class of filamentous fungi also accommodates associates of other arthropods such as bark beetles and mites (Spatafora et al. 2006, Zhang et al. 2006, De Beer et al. 2013. Wingfield et al. 2017). More than 150 species residing in the orders Ophiostomatales, Microascales and Hypocreales are known mutualists of these arthropods (Kasson et al. 2013, Kolařik & Jankowaik 2013, De Beer et al. 2013, Wingfield et al. 2017). Unlike ambrosia beetles, bark beetles and mites are not obligately dependent on their fungal mutualists for survival, but in many cases, the fungi form an integral part of their diet (Six 2012, Hofstetter et al. 2013). To ensure that they never lose their fungal partners, these different arthropods have evolved specialized exoskeletal structures in which they maintain their fungal associate during dispersal (Hulcr & Stelinski 2017). In turn, the fungal mutualists have also evolved morphological adaptations to ensure effective arthropod dispersal and survival within the insect galleries (Kolařik & Jankowaik 2013). These different morphological characters are sufficiently distinctive that, prior to the ready availability of DNA sequence-based phylogenetic inference, researchers have mistakenly treated various genera and even sub-classes of the Sordariomycetes as taxonomically related (Spatafora & Blackwell 1994, De Beer et al. 2013).

Convergent evolutionary morphological traits can be found in all Kingdoms of life, where distantly related species share a common ecological lifestyle. While morphological convergence is easily detected by simple observational methods, genetic and genomic convergence is less well understood. Advances in genome sequencing and the analytical tools used to study their evolution provide new opportunities to gain insight into these traits (Hall 2013, Arbuckle & Speed 2016).

In this study, we considered the genomic patterns of adaptation to an arthropod-associated lifestyle in Sordariomycete fungal species. This was achieved by analysing and comparing the genomic patterns of approximately 45 arthropod-associated species residing in the three different Sordariomycete orders with their related non-symbiotic species (**Table 1**). In doing so, we discovered that long time co-evolution with their various arthropod partners has resulted in convergent losses across some major gene families involved in nutrient acquisition and host-pathogen interaction.

3. Materials and Methods

3.1. Taxon sampling

Phylogenomic and comparative genomic analyses of 110 species of Sordariomycetes were performed. The analyses included the genomes of 45 species of arthropod symbionts that reside in the Ophiostomatales, Microascales, and Hypocreales. Additionally, the genomes of 62 other representative Sordariomycete species representing several diverse lifestyles and three Dothidiomycete outgroup species were also included.

Genomes were downloaded from the JGI fungal genome portal MycoCosm (http://jgi.doe.gov/fungi) and NCBI Genbank (https://www.ncbi.nlm.nih.gov/genbank/). Where necessary permission was obtained from corresponding principle investigators to utilize unpublished data.

3.2. Additional genome sequencing

Genome sequences for two species of Annulatascaceae were generated in this study. Details regarding the sequencing and assembly process are presented in **Supplementary File 1**. These genomes will be deposited in NCBI GenBank and the accession numbers provided in the reference column of **Table 1**.

3.3. Genome annotation

Genome annotation was carried out for species where annotation data were not available. These included most of the Ceratocystidaceae and Ophiostomataceae genomes, as well as the newly sequenced genomes of *Natantiella ligneola* and *Xylomeasma sordida*. The MAKER V2.31.8 (Cantarel *et al*. 2008) genome annotation pipeline was used for the annotation process. GENEMARK-ES V4.38 (Lomsadze et al. 2005) with the "fungus" option enabled (using self-training; Ter-Hovhannisyan et al. 2008) and AUGUSTUS V3.2.2. (using species models optimized for *Neurospora crassa*; Stanke et al. 2006) were used as gene predictors. Before the gene model prediction step, the genomes were masked against specific repeat libraries generated with REPET (Quesneville et al. 2005, Flurte et al. 2011). Available proteome datasets from closely related species, where available, and SWISS-PROT protein database (https://www.uniprot.org/, Access date 2018-08-17) were provided to MAKER as protein homology

evidence. Quality of the annotations in terms of covering organisms gene space was assessed using the BENCHMARKING UNIVERSAL SINGLE-COPY ORTHOLOGS (BUSCO V2.0.1) tool (Simão et al. 2015).

3.4. Species tree analysis

3.4.1. Supermatrix analysis

All genomes were assessed using the BENCHMARKING UNIVERSAL SINGLE-COPY ORTHOLOGS (BUSCO V2.0.1) tool against the Ascomycota dataset (Simão et al. 2015). Shared single-copy orthologous genes were then identified and their peptide sequences were extracted using the pipeline available in the **Supplementary File 1**. The individual protein datasets for each of the 315 identified shared single-copy orthologous genes were aligned using PRANK. The aligned datasets were trimmed using GBLOCKS V0.91B (Castresana 2000) with the allowed gap parameter set to half to eliminate poorly aligned and highly divergent regions. Resulting alignments were subjected to permutation tail probability (PTP) testing (Archie 1989) using PAUP V4.0 (Swofford 1998) to evaluate their phylogenetic signal. Two datasets that failed the PTP test (P-value > 0.001) were removed from further analysis due to lack in their phylogenetic signal. The remaining datasets were concatenated into a supermatrix containing 114,626 positions using FASCONCAT V1.04 (Kück & Meusemann 2010). The supermatrix was run through PARTITIONFINDER V2.1.1 (Lanfear et al. 2016) using RCLUSTER search (Lanfear et al. 2014) and the PF2 option set to RaxML (Stamakis 2006) to determine the best-fit partitioning scheme. A maximum likelihood tree was inferred for the partitioned supermatrix using RaxML PTHREADS-AVX2 with the model specified according to the best scheme identified for the partitioned dataset by PARTITIONFINDER. The phylogeny was tested using 100 bootstrap repetitions. The three Dothidiomycete species (ABRA, PFIJ and SNOD) were specified as outgroup taxa.

3.4.2. Supertree analysis

Since supermatrix phylogenomic reconstruction using concatenation can introduce bias due to genes with strong phylogenetic signal (Shen et al. 2017), supertree analysis using STAG was also performed. Unlike concatenation approaches that preferably rely on single-copy orthologous genes, supertree reconstruction using STAG incorporates both single-copy and multi-copy orthologous gene families

(Emms & Kelly 2018b). To identify orthologous gene families within the genomes of all species considered, protein clustering was conducted using ORTHOFINDER v2.3.1 (Emms & Kelly 2018a, 2019) with inflation factor of 2.5. Orthologous groups shared between more than three species were then selected for further analysis. Protein sequences from each of the selected orthologous groups were extracted and aligned using PRANK (Löytynoja 2014). The aligned datasets were treated with TRIMAL (Capella-Gutiérrez et al. 2009) using the -automated1 option to remove ambiguous aligned positions. Maximum likelihood tree construction was carried out for each individual dataset using IQ-TREE (Nguyen et al. 2015) with best substitution model automatically selected and 1000 ultrafast bootstrap iterations. Finally, STAG was used to construct a supertree from all individual gene trees obtained for each of the orthologous groups.

3.5. Molecular dating

A concatenated alignment of 50 genes was used to estimate the divergence dates of taxa in the Sordariomycetes with BEAST V2.5 (Bouckaret et al. 2019). The most recent common ancestor of the Hypocreales was calibrated at 180 million years (sampling between 170–190 mya), the *Diaporthe ampelina* speciation (based on fossil evidence) at 136 million years (sampling between 126–146 mya) and the MRCA of the Sordariomycetes at 290 million years (sampling between 260–320 mya) (Réblová & Štěpánek 2009, Beimforde 2014, Pérez-Ortega et al. 2016). The dating analyses were constrained to the topology of the STAG tree, and run for 5,685,000 generations, with a Blosum model of evolution for each partition and a strict clock. Convergence of all priors was visualised in TRACER V1.7 (Rambaut 2018) and 5,120 trees were summarised with TREEANNOTATOR, part of the BEAST V2.5 package.

3.6. CAZyme identification

Putative Carbohydrate Active Enzymes (CAZymes) were identified and annotated using the dbCAN meta server (database for automated CAZyme annotation: bcb.unl.edu/dbCAN2) and the HMMER, DIAMOND and Hotpep identification tools (Zhang et al. 2018). A protein was considered as a true CAZyme if it were predicted by at least two of the three identification tools. Identified CAZymes were first grouped based on their different families (GT=Glycosyl Transferases; GH=Glycoside Hydrolases;

PL=Polysaccharide Lyases; CE= Carbohydrate Esterases; AA=Auxiliary Activities Enzymes; CBM=Carbohydrate Binding Modules) and then individual family classes were investigated further based on their previously identified roles in wood decay (E.g. lignin degradation: AA1, AA2; Crystalline cellulose degradation: GH6, GH7, AA9).

3.7. Lipase

Putative lipases and cutinases were identified using a HMMER search (e-value = 1e-04) against the LIPASE ENGINEERING DATABASE (Release 3.0: http://www.led.uni-stuttgart.de/). Detailed annotation pipelines for peptidase identification can be found in the **Supplementary File 1**.

3.8. Peptidase identification

Putative peptidases were identified by doing a BLASTp search (e-value = 1e-04) against full length sequences contained within the MEROPS DATABASE (Release 12.0: https://www.ebi.ac.uk/merops/) (Rawlings et al. 2017). Detailed annotation pipelines for peptidase identification can be found in the **Supplementary File 1**.

3.9. Secondary metabolite gene cluster identification

To identify putative gene clusters involved in secondary metabolism, we used antiSMASH V4.2.0 analysis of the whole genomes (Blin et al. 2017). Analysis was conducted using default settings and the taxon option set to "fungi". Detailed annotation pipelines for secondary metabolite gene cluster analyses can be found in the **Supplementary File 1**.

3.10. Small, secreted protein identification

To identify and annotate putative secreted proteins, all proteins that contained a secretion signal (as determined using SIGNALP v4.0; Petersen et al. 2011) were first identified. All the proteins that contained no transmembrane domains (as determined by TMHMM; Krogh et al. 2001) were then identified. Finally, all proteins that form part of the secretory pathway (as determined by TARGETP v1.0, Emanuelsson et al. 2000) were determined. A protein was considered a true secreted protein if it was recognized by at least two of the annotation tools i.e. contains a secretion signal, contains no

transmembrane domains, and forms part of the secretory pathway. The identified secreted proteins were screened for those smaller than 200 amino acids, which in this study were considered as small, secreted proteins. Detailed annotation pipelines used for the identification of secreted proteins can be found in the **Supplementary File 1**.

4. Results and Discussion

4.1. The origin of arthropod symbiotic lineages in the Sordariomycetes

The Sordariomycetes represent one of the largest fungal Classes including a wide diversity of species displaying numerous different lifestyles and niche adaptations. To infer the origin of the arthropod mutualistic fungi in this Class, we generated a species-tree based on phylogenomic analyses of protein sequences for a total of 110 species, 45 of which are arthropod-associated species, using both supertree and supermatrix methods. Details on the different approaches and comparisons of the results are presented in the supplementary material. The resulting phylogenies obtained using the different analyses (**Figure 1; Supplementary Figures 1-2**) were highly congruent with each other and with those produced in previous studies of the Class (De Beer & Wingfield 2013, Maharachchikumbura et al. 2016). All 45 species of arthropod mutualistic fungi investigated in this study resided in the orders Ophiostomatales, Microascales and Hypocreales; each of which are treated individually below.

4.1.1. Microascales

Twenty-one species in the Ceratocystidaceae and Gondwanamycetaceae were included in our analyses. The phylogeny grouped these families as sister lineages, and together they formed a monophyletic clade in the Microascales. These families share morphological adaptation to arthropod dispersal, which suggests a common origin of arthropod mutualism that predates the divergence of the more recent sub-lineages. Our molecular clock analyses (**Figure 1)** suggest that these families represent the oldest arthropod associated lineage in the Sordariomycetes, with the most recent common ancestor estimated around 220 mya.

4.1.2. Ophiostomatales

In our analyses, the Ophiostomatales formed a monophyletic clade that shared an evolutionary relationship with members of the Annulatascaceae. This phylogenetic placement has also been shown in previous studies (Réblová 2006, Réblová & Štěpánek 2009). Members of the Annulatascaceae are saprophytes of decaying wood, with sexual morphologies like those of species residing in the Ophiostomataceae and Ceratocystidaceae, which are commonly seen as a morphological adaptation to arthropod dispersal. Given the shared morphological adaptation, it is probable that members of the Annulatascaceae also rely on arthropods for their dispersal. This suggests that the sexual structures consisting of long necked perithecia producing ascospores in sticky masses is an adaptation for insect dispersal shared between Annulatascaceae and Ophiostomataceae around 200 mya.

Our analyses also suggest that the first association between Ophiostomatalean fungi and bark beetles evolved at around 156 mya. From that point, some genera such as *Graphilbum, Ophiostoma, Grosmannia* and *Leptographium* retained their symbiotic relationships with bark beetles, whereas other genera such as *Ceratocystiopsis* and *Raffaelea* later evolved obligate associations with ambrosia beetles. The remaining genera including *Sporothrix, Fragosphaeria, Hawksworthiomyces,* and *Esteya* evolved entirely different lifestyles. These variations in lifestyle make the genomic patterns we discuss next somewhat more obscure for some of the Ophiostomataceae, for example among species of *Hawksworthiomyces*, but overall, for genera that maintained their arthropod symbioses, similar patterns can be found. The phylogenetic patterns emerging from the present analyses closely resemble those of Vanderpool et al. (2018) produced by MLSA.

4.1.3. Hypocreales

There are two genera of the Hypocreales accommodating species identified to have associations with bark and ambrosia beetles. One is *Geosmithia*, a genus that accommodates several common but understudied species of both bark and ambrosia beetle symbionts (Spatafora & Blackwell 1994, Kolařík & Kirkendall 2010). Three *Geosmithia* species, *G. flava, G. morbida,* and *G. putterilli*, were included in our analyses. The emerging phylogeny grouped these species close to *Acremonium chrysogenum* and

Stanjemonium grisellum, in the Bionectriaceae. This result is like that produced by Schuelke et al. (2017). Because none of the other Bionectriaceae species are known to have adaptation to arthropod dispersal, the symbiosis with bark and ambrosia beetles is restricted only to members of *Geosmithia*, which was estimated to have emerged around 52 mya.

The second beetle associated group in the Hypocrealeas is found for some species of *Fusarium*. These symbiotic species form a unique clade in the *F. solani* complex, which is referred to as the Ambrosia Fusarium Clade (AFC) (Kasson et al. 2013, Lynn et al. 2020). Our analyses included the plant pathogenic symbiont of the ambrosia beetle *Euwallaceae fornicatus*, *F. euwallaceae*, which was the only species with an available genome sequence from this clade at the time of our study (Ibarra-Laclette et al. 2005, Freeman *et al*. 2013) Similar to other phylogenetic investigations, our analyses placed this species as a sister lineage to *F. solani* (Kasson et al. 2013, Freeman et al. 2013). Based on their placement and monophyletic grouping in the genus, the association between AFC *Fusaria* and ambrosia beetles likely arose only once during a single evolutionary event in the history of the genus. Our molecular clock analysis also suggests that this is the most recent Sordariomycete lineage to have evolved an association with a beetle vector, having last shared a common ancestor with other *Fusarium* spp. around 15 mya.

4.2. Arthropod mutualists have a reduced genome size and gene content

Recent investigations have reported that micro-organisms with highly specialized or symbiotic lifestyles often have reduced genome size and gene content (Lawrence 2005, McCuthceon & Moran 2012). When we compared the genome sizes of arthropod symbiotic species in this study with those of the other Sordariomycetes, we found a similar trend, with these fungi frequently having smaller genome sizes than their relatives. In addition to having smaller genome sizes, they also have reduced gene numbers. This genome reduction can be attributed to a phenomenon known as the minimal genome concept (Mushegian 1999, McCuthceon & Moran 2012), which suggests that a micro-organism living in symbiosis with another organism will maintain only the genes essential for life with the remainder being lost over time.

Overall, the genomes of arthropod symbiotic species range from 19.5Mb to 40Mb in size and contain between 5296 and 10816 genes (**Table 1**). Although this might appear as a substantial level of variation, the variability in the genomes of these symbiotic fungi can be explained by a number of factors including: (1) the length of time that a symbiosis between a fungal lineage and an arthropod vector has existed; (2) the nature of the association between the fungus and its vectors; and (3) the host range of the fungus-vector systems. For example, species of *Ceratocystiopsis* have some of the smallest genomes so far identified in the Sordariomycetes [around 21Mb]. These fungi are mycangial symbionts of a small number of Scolytine beetles that live in the outer bark-phloem interface of *Pinus* spp. (Moser et al. 1995, Hsiau & Harrington 1997, Zipfel et al. 2006, Six 2019). This restriction in host range, arthropod vector, and unique niche locality could explain why these species have such small genomes. In contrast, species of *Raffaelea sensu lato*, have some of the largest genomes amongst arthropod symbiotic fungi. Although all species of this genus are mycangial symbionts of ambrosia beetles (Dreaden et al. 2014), phylogenomic analyses (**Figure 1**) split this genus into three distinct lineages, which we define as the *R. lauricola, R.arxii* and *R. quercus* complexes. When these different clades are compared, it is evident that species in the *R. lauricola* and *R. arxii* complexes have larger genomes with wider variety of genes (Caballero et al. 2019) than those in the *R. quercus* complex. However, several species in the former two complexes have been found to associate with multiple beetle vectors (Ploetz et al. 2013, Carillo et al. 2014, Saucedo-Carabez et al. 2018) and are consequently also vectored to a larger repertoire of hosts. These species would therefore require a larger number of genes to be able to contend with the extensive array of host defence molecules and available nutritional substrates.

The only exception to our findings of genome reduction and gene loss was *F. euwallacace*, which has a genome similar in size and gene number to those of its non-symbiotic relatives. Although this species is known only to associate with a single ambrosia beetle species, its vector is highly polyphagous. In a recent study by Eskalen et al. (2013) the authors were able to recover *F. euwallaceae* from approximately 180 different tree species that showed signs of attack by *E. fornicatus*. Together with this large host range of its vector, the very recently evolved arthropod association of *F. euwallacace*

could explain the fact that little to no genomic change observed in this species compared to its closely related non-insect-vectored associates.

4.3. The reduction in gene families is involved in pathogenicity

Secondary metabolites, virulence factors and other effectors are all molecules involved in inter-species interactions, niche adaptation, pathogenicity, and nutrient acquisition. When investigating changes in genome structure between different communities, such as in this study of arthropod versus non-arthropod symbiotic Sordariomycete species, it becomes possible to compare differences in the repertoires of functional enzymes produced by different species displaying different lifestyles (Ohm et al. 2012, Lah et al. 2017, Caballero et al. 2019). This is because expansion and contraction events in protein and enzyme families can provide valuable insights into the resource utilization, ecology, and community role of a particular group of organisms (Whinston & Taylor 2016). For this reason, we annotated the proteome for all the species included in the phylogenomic analyses. Results of these annotations showed that each of the protein and enzyme families investigated have undergone losses in gene number in the Sordariomycetes that have symbiotic relationships with arthropods (**Figure 2; Supplementary Tables 1-6**).

4.3.1. Carbohydrate active enzymes (CAZymes)

Enzymes involved in complex carbohydrate metabolism are commonly known as CAZymes. As all organisms require complex carbohydrates to survive, these enzymes are essential for their roles in synthesis, modification, and degradation of these molecules (Cantarel et al. 2008a, Riley et al. 2014, Druzhinina et al. 2018, Zhang et al. 2018). Annotation of the CAZyme repertoires of the various Sordariomycete species considered in this study revealed homologs from 172 CAZyme families with representatives of all six known classes (13 AA, 16 CBM, 12 CE, 86 GH, 34 GT, and 11 PL) (**Supplementary Tables 2 & 7**). Overall, we found that the arthropod mutualistic species have lower numbers of CAZymes (min 167 [CBRE], max 380 [RALB], average 230) than other Sordariomycete species included in our analyses (min 197 [TANT], max 680 [ILYO], average 415]. Although there were high levels of variability in all six classes of CAZymes, when we compared the arthropod

symbiotic and non-arthropod symbiotic Sordariomycetes, the greatest variability between species was observed within the GH, GT and AA classes (**Table 2**). These all contain families that are involved in the breakdown of plant cell-wall constituents.

Because arthropod symbionts are mostly found on woody plants or substrates, we were particularly interested in investigating CAZyme families involved in wood degradation. To achieve this goal, 45 CAZyme families that are known to be involved in the degradation of woody constituents were considered (**Supplementary Table 8**). By screening the CAZyme families identified by dbCAN2, homologs of 37, 31 and 28 of these families were found in the Ophiostomatales, Microascales and *Geosmithia,* respectively. However, these families appear to have undergone a reduction in gene number within the genomes of the arthropod symbiotic Sordariomycete species (**Figure 2**). This finding was not unexpected as it has been shown previously that Ophiostomatoid fungi are not involved in the breakdown of these compounds (Valiev *et al* 2009, Six 2019). This is supported by the findings of Skelton et al. (2019) who showed that fungal symbionts of bark and ambrosia beetles are not involved in wood decay, but rather slow down the decay process by out-competing wood decay fungi. Our results substantiate these findings and suggest that the arthropod symbiotic Sordariomycetes are not actively involved in wood decay.

4.3.2. Lipases and Peptidases

Fungi are known to produce an arsenal of hydrolytic enzymes that function in the breakdown of plant cell wall constituents and host signalling molecules. Lipases primarily act on the outside of the plant, breaking down the cuticle, aiding in invasion into a host (Souza *et al*. 2015, Da Silva 2017). Based on their a/b hydrolase folds, three classes of lipases are recognized (GX, GGGX, and Y), each containing several superfamilies. Annotation of these enzymes in the fungi considered in this study using the LED database identified candidates belonging to all three classes and 29 superfamilies (5 GGGX class, 4 Y class, and 20 GX) (**Supplementary Table 3**). Many of these superfamilies were present in low numbers in some species with only 11 of the 29 superfamilies having homologs in all species. Of these, superfamilies abH03, abH04, abH08 and abH09 were the most abundant in all species.

Peptidases are involved in both the intracellular and extracellularly breakdown and modification of proteins and their amino acid constituents (Souza *et al*. 2015, Da Silva 2017), disrupting signalling and aiding in nutrient acquisition. Based on their hydrolytic capabilities, seven peptidase types have been described (Aspartic [A]; Asparagine [N]; Cysteine [C]; Glutamic [G]; Metallo [M]; Serine [S]; and Threonine [T]). Peptidase annotation using the MEROPS DATABASE identified homologs of all catalytic types within the investigated Sordariomycetes (**Supplementary Table 4**). The most abundant peptidases identified were Serine peptidases, along with a consortium of Metallo, Cysteine, Aspartic and Threonine peptidases. Homologs to 109 different families were identified [6=A; 29=C; 1=G; 10=I; 37=M; 2=N; 20=S; 4=T], 40 of which had at least one representative in all species included (**Supplementary Table 9**).

The large variability in lifestyle for species in the Sordariomycetes also implies that the hydrolytic enzymes they produce are also highly variable. However, when we compared the arthropod symbiotic species with non-symbiotic Sordariomycetes, the plant pathogenic species, such as species of *Ilyonectria* and *Fusarium*, had much higher numbers of these enzymes in their genomes. However, the overall differences in gene number for both lipases and peptidases can be attributed to a reduction in a limited number of families. For example, peptidase families A01, S08, S09, S10 and S33 are present in very low numbers in the genomes of arthropod symbiotic species compared to the non-symbiotic species.

In general, we found that the lipase and protease families reduced in arthropod mutualistic species were those that act in the extracellular space and are involved in pathogenicity. Because these fungi are rarely found colonizing the extracellular spaces of the host, a loss of the genes required to survive in this region would not be surprising. These results also explain why, in most cases, the arthropod symbiotic species require wounds (Heath *et al* 2009a, 2009b) to infect their hosts, consistent with the fact that these fungi have reduced efficacy in penetrating the plant cell wall. In part, the results also highlight the importance of the arthropod vectors in the success of their symbiotic fungi, because they not only produce wounds allowing entry into the host, but also aid the fungi in bypassing the first line in plant defences.

4.3.3. Secondary metabolites

Secondary metabolites such as pigments, phytotoxins and siderophores are low-molecular weight molecules produced by organisms that are not involved in growth but are involved in adaptation to different environments, the virulence of plant pathogens, and in interspecies interaction. Genes encoding these metabolites are often found in clusters within the genome, hence they are called secondary metabolite gene clusters (SMGCs) (Keller *et al*. 2005, Brakhage 2012, Hoffmeister & Keller 2007, Kjærbølling et al. 2018) When we screened the genomes of the Sordariomycetes considered in this study for the overall presence of SMGCs we found high variably in SMGC encoding genes in the species investigated (**Supplementary Table 5**). However, consistent with our other results, we found that the arthropod mutualistic species have some of the lowest numbers of SMGCs among the Sordariomycetes, ranging from seven to 26 [average 13], whereas the non-mutualistic species had between 14 and 63 SMGCs [average 29].

Although secondary metabolites are often considered as non-essential for life, in filamentous fungi they play an important role in lifestyle adaptation (Brakhage 2012). In general, the abundance of SMGCs in the genomes of the Sordariomycete fungi were quite disparate, with higher levels of similarity was found in species with close phylogenetic relationships. This pattern of distribution is not uncommon and has been shown before for species of Dothidiomycetes (Ohm et al. 2012). However, numerous previous studies investigating SMGCs have shown that even closely related species can have vastly different assemblages of these clusters in their genomes (Kjærbølling et al. 2018, Vesth *et al* 2018). Despite recent efforts in studying SMGCs, the evolutionary drivers behind their variability remains unclear (Lind et al. 2017) but as these molecules are involved in processes such as defence, nutrition and communication many environmental factors can influence their presence within a species genome (Vesth *et al* 2018).

Primarily the losses we observed in the investigated arthropod mutualistic fungi were from three groups of SMGCs: nonribosomal peptide synthetases (NRPS), type 1 polyketide synthases (T1PKS), and terpene synthases. As the names of these groups suggest, each are involved in the production of a different class of biologically active compound (nonribosomal peptides, polyketides, and terpenoids,

respectively) (Bills & Gloer 2106). A reduction in the genes encoding these multimodular synthesis enzymes in the genome of an organisms, like what we observed for the arthropod mutualistic species investigated, would suggest a reduction in the bioactive compounds they can produce. Based on the roles that these compounds play, this reduction suggests an overall reduced capability of these species in adapting to different or changing environments.

4.3.4. Small secreted proteins (SSPs)

Small secreted proteins are important molecules involved in defence and virulence in filamentous fungi. These small peptides (200 amino acids or less) act as avirulence factors and play a key role in supressing host innate immune responses (Rep 2005). Identification of SSPs encoded in the genomes of all the Sordariomycetes investigated revealed the same trend seen for the other annotations carried out in this investigation. When comparing the various Sordariomycete species we found that the arthropod mutualists encode some of the lowest (**Supplementary Table 6**) numbers of SSPs whereas non-mutualistic plant pathogens encode the highest. This finding is similar to that of Ohm et al. (2012), who found that found that plant pathogenic species have higher numbers of SSPs than saprotrophic species. Similar to many of our other results, we found that small secreted proteins make up a smaller proportion of the total secreted proteins in arthropod symbiotic fungi compared to other members of the Sordariomycetes (**Figure 2B**).

5. Conclusions

Species of Sordariomycete fungi are highly diverse, occupy a variety of niches and utilize many different lifestyles. Various lineages in this class have evolved convergent symbiotic relationships with arthropod vectors and this lifestyle was the focus of the present investigation. Comparative genomic analyses of more than 100 species of Sordariomycete fungi revealed that these symbionts have reduced genome sizes with lowered gene content. Most of these arthropod symbiotic species are not regarded as biotrophic pathogens, being unable to infect a host without the presence of a wound, often induced by their vectors (Heath 2009a, b, Wingfield et al. 2017). This was reflected in their genomes as a reduction in gene content resulting from losses of many gene families. Therefore, this study provides

valuable insights into the genomic patterns resulting from the evolution of an arthropod mutualistic lifestyle and how the changes in the genome affect the pathogenicity of these fungi.

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Table 1. Species used in the analyses

Species name	Abbrev.	Origin of isolate	Lifestyle	Size (Mb)	Complete* (%)	Reference
Acremonium alcalophilum	AACR	Pig Manure	Alkaliphile/Saprophyte	54,3	97,7	JGI
Alternaria brassicola	ABRA	*Brassica sp.*	Plant pathogen	30,3	90,5	Ohm et al. (2012)
Acremonium chrysogenum	ACHR	Seawater	Saprophyte	28,6	99,3	Terfehr et al. (2014)
Apiospora montagnei	AMON	Detritus	Saprophyte	47,7	98,4	JGI
Ambrosiella xylebori	AXYL	Ambrosia beetle gallery	Arthropod symbiont	27,2	97,5	Vanderpool et al. (2018)
Beauveria bassiana	BBAS	Insect	Entomopathogen	33,6	98,7	Xiao et al. (2012)
Bretziella fagacearum	BFAG	*Quercus rubra*	Arthropod symbiont	26,6	96,6	Wingfield *et al*. (2016b)
Colletotrichum acutatum	CACU	*Pinus radiata*	Plant pathogen	49,1	98,2	Han et al. (2016)
Ceratocystis adiposa	CADI	*Saccharum officinarum*	Plant pathogen	28,3	97,7	Wingfield et al. (2016a)
Ceratocystis albifundus	CALB	*Terminalia sericea*	Arthropod symbiont	26,7	97,7	Van Der Nest et al. (2014b)
Ceratocystiopsis brevicomis	CBRE	Western pine beetle	Arthropod symbiont	19,5	91,8	Vanderpool et al. (2018)
Ceratocystis eucalypticola	CEUC	Wounded Eucalyptus	Arthropod symbiont	30,7	97,4	Wingfield et al. (2015b)
Ceratocystis fimbriata	CFIM	*Ipomoea batatas*	Arthropod symbiont	29,9	97,7	Wilken et al. (2013)
Chaetomium globosum	CGLO	Stored cotton	Saprophyte	34,3	91,3	Cuomo *et al*. (2015)
Colletotrichum graminicola	CGRA	Maize	Plant pathogen	50,9	99,0	O'Connell et al. (2012)
Colletotrichum higginsianum	CHIG	*Brassica campestris*	Plant pathogen	50,8	99,1	Zampounis et al. (2016)
Coniochaeta ligniaria	CLIG	*Picea abies*	Endophyte	43,2	95,7	Jimènes et al. (2017)
Ceratocystis manginecans	CMAN	*Prosopsis cineraria*	Arthropod symbiont	31,4	97,3	Van Der Nest et al. (2014a)
Corollospora maritima	CMAR	Detritus	Saprophyte/Marine	36,9	98,0	JGI
Cordyceps militaris	CMIL	Caterpillar	Entomopathogen	32,2	98,4	Zheng et al. (2011)
Ceratocystiopsis minuta	CMIN	*Ips cembrae*	Arthropod symbiont	21,2	94,0	Wingfield et al. (2016a)
Cryphonectria parasitica	CPAR	American Chestnut	Plant pathogen	43,8	98,6	JGI
Ceratocystis platani	CPLA	*Platanus* sp.	Arthropod symbiont	29,1	97,1	NCBI unpublished
Ceratocystis smalleyi	CSMA	*Carya cordiformis*	Arthropod symbiont	25,7	97,0	Wingfield et al. (2018a)
Diaporthe ampelina	DAMP	Grapevine	Endophyte	50	97,8	Morales-Cruz et al. (2015)
Daldinia eschscholzii	DESC	*Myroxylon balsamum*	Endophyte	37,4	98,4	Wu *et al*. (2017)
Davidsoniella virescens	DVIR	*Acer saccharum*	Arthropod symbiont	33,4	97,3	Wingfield et al. (2015b)
Endoconidiophora laricicola	ELAR	*Larix* sp.	Arthropod symbiont	32,7	97,4	Wingfield et al. (2016a)
Eutypa lata	ELAT	*Vitis vinifera* cv. “Cremson”	Plant pathogen	53,8	97,0	Blanco-Ulate et al. (2013a)
Endoconidiophora polonica	EPOL	*Picea abies*	Arthropod symbiont	32,4	97,5	Wingfield et al. (2016a)

Species name	Abbrev.	Origin of isolate	Lifestyle	Size (Mb)	Complete* (%)	Reference
Esteya vermicola	EVER	*Scolytus intricatus*	Nematophyte	34	97,5	Vanderpool et al. (2018)
Fusarium euwallaceae	FEUW	Kuroshio shot hole borer	Arthropod symbiont	48,2	98,9	Ibarra-Laclette *et al*. (2017)
Fusarium fujikuroi	FFUJ	*Saccharum officinarum*	Plant pathogen	43,8	99,0	Wiemann et al. (2013)
Fusarium graminearum	FGRA	Wheat kernels	Plant pathogen	36,2	98,8	Cuomo *et al*. (2007)
Fusarium oxysporum	FOXY	*Cardamine hirsute*	Plant pathogen/ Saprophyte	55,7	99,3	Ma et al. (2010)
Fragosphaeria purpurea	FPUR	*Fagus sylvatica*	Saprophyte	34,8	98,3	Vanderpool et al. (2018)
Fusarium verticillioides	FVER	Maize	Plant pathogen	41,7	98,8	Ibarra-Laclette *et al*. (2017)
Grosmannia clavigera	GCLA	*Dendroctonus ponderosae*	Arthropod symbiont	29,1	97,8	DiGuistini *et al*. (2011)
Grosmannia cucullata	GCUC		Arthropod symbiont	23,5	98,4	Currently unpublished
Geosmithia flava	GFLA	*Castenea sativa*	Arthropod symbiont	29,5	99,0	Schuelke et al. (2017)
Graphilbum fragrans	GFRA	*Hylastes angustatus*	Arthropod symbiont	34,3	98,1	Wingfield et al. (2015b)
Grosmannia galeiformis	GGAL	*Pinus sylvestris* infested with *Tomicus piniperda*	Arthropod symbiont	26,4	94,8	Wingfield *et al*. (2018b)
Geosmithia morbida	GMOR	*Juglans californica*	Arthropod symbiont	26	98,9	Schuelke et al. (2017)
Grosmannia penicillate	GPEN	*Ips amitinus*	Arthropod symbiont	26,3	97,2	Wingfield et al. (2016b)
Grosmannia piceaperda	GPIC		Arthropod symbiont	28 ,8	98,3	Currently unpublished
Geosmithia putterilli	GPUT	*Juglans californica*	Arthropod symbiont	30	99,3	Schuelke et al. (2017)
Huntiella decipiens	HDEC	*Staphilinid* sp. infesting *Eucalyptus saligna*	Arthropod symbiont	26,6	96,6	Wingfield et al. (2017)
Hawksworthiomyces lignivorus	HLIG	Eucalyptus pole at soil level	Saprophyte	43,8	97,6	Wingfield et al. (2017)
Huntiella moniliformis	HMON	*Eucalyptus grandis*	Arthropod symbiont	25,4	96,5	Van Der Nest et al. (2014a)
Huntiella savannae	HSAV	*Acacia nigrescen*	Arthropod symbiont	28,5	96,6	Van Der Nest et al. (2015)
Hypoxylon sp.	HYPO	*Neea floribunda*	Saprophyte	47,6	97,8	Wu *et al*. (2017)
Ilyonectria europaea	IEUR	*Arabidopsis thaliana*	Plant pathogen	62,3	98,9	JGI
Ilyonectria sp.	ILYO	*Populus deltoides*	Plant pathogen	63,2	98,8	JGI
Ilyonectria robusta	IROB	*Populus trichocarpa*	Plant pathogen	59,7	98,9	JGI
Knoxdaviesia capensis	KCAP	*Protea longifolia* infructescences	Arthropod symbiont	35,5	97,0	Aylward et al. (2016)
Knoxdaviesia proteae	KPRO	*Protea repens* infructescences	Arthropod symbiont	35,1	94,2	Aylward et al. (2016)
Leptographium douglasii	LDOU		Arthropod symbiont	31	98,1	Currently unpublished
Leptographium lundbergii	LLUN	*Pinus Sylvestris*	Arthropod symbiont	26,5	98,1	Wingfield et al. (2015a)
Leptographium procerum	LRPO	*Pinus resinosa*	Arthropod symbiont	28,5	91,0	Van Der Nest et al. (2014b)

Species name	Abbrev.	Origin of isolate	Lifestyle	Size (Mb)	Complete* (%)	Reference
Leptographium wageneri var pseudotsuga	LWAP		Arthropod symbiont	38,5	97,4	Currently unpublished
Metarhizium acridum	MACR	Locust	Entomopathogen	38,1	98,3	Gao et al. (2011)
Magnaporthe grisea	MGRI	Rice pathogenic strain	Plant pathogen	41,5	98,5	Dean *et al*. (2005)
Metarhizium robertsii	MROB	*Conoderus* sp.	Entomopathogen	39	98,5	Gao et al. (2011)
Myceliophthora thermophila	MTHE	Soil	Saprophyte/Thermophile	38,7	98,9	Berka *et al*. (2011)
Microascus trigonosporus	MTRI	Skin lesion	Animal pathogen	36	97,8	JGI
Neurospora crassa	NCRA	Inbred genetically stable reference wild-type strain	Saprophyte	41	99,3	Galagan *et al*. (2003)
Neurospora discreta	NDIS	Unknown	Saprophyte	37,2	98,7	JGI
Fusarium solani	NHAE	Third generation cross between a pea isolate and potato field soil isolate	Plant pathogen	51,2	99,2	Coleman *et al*. (2009)
Neurospora hispaniola	NHIS	Burned vegetation	Saprophyte	40,8	99,0	JGI
Natantiella ligneola	NLIG	Wood of water-soaked branch	Saprophyte	35,2	96,9	This study
Ophiostoma bicolor	OBIC	*Picea abies*	Arthropod symbiont	25	97,6	Lah et al. (2017)
Ophiostoma ips	OIPS	*Pinus taeda*	Arthropod symbiont	26	98,5	Wingfield et al. (2017)
Ophiostoma novo-ulmi	ONOV	Elm	Arthropod symbiont	31,8	99,1	Forgetta *et al*. (2013)
Ophiostoma piceae	OPIC	*Pinus contorta*	Arthropod symbiont	32,5	98,8	Haridas *et al*. (2012)
Ophiostoma ulmi	OULM		Arthropod symbiont	31,1	98,2	Khoshraftar et al. (2013)
Phaeoacremonium aleophilum	PALE	Margin of grapevine (*Vitis vinifera* cv. “Thomson”)	Plant pathogen	47,4	98,8	Blanco-Ulate et al. (2013b)
Podospora anserina	PANS	Dung	Saprophyte	35,5	99,1	Espagne et al. (2008)
Pseudocercospora fijiensis	PFIJ	*Musa* sp.	Plant pathogen	73,7	98,3	Arango Isaza et al. (2016)
Raffaelea aguacate	RAGU	*Persea americana*	Arthropod symbiont	35,2	98,5	Vanderpool et al. (2018)
Raffaelea albimanens	RALB	*Platypus externedentatus* in *Ficus sycomo*	Arthropod symbiont	39	98,6	Vanderpool et al. (2018)
Raffaelea ambrosiae	RAMB	*Platypus cylindrus* gallery in *Quercus*	Arthropod symbiont	40,5	98,0	Vanderpool et al. (2018)
Raffaelea arxii	RARX	*Xyleborus torquatus* on *Cussonia umbellif*	Arthropod symbiont	36,7	98,3	Vanderpool et al. (2018)
Raffaelea lauricola	RLAU	Unknown	Arthropod symbiont	34,3	98,3	Vanderpool et al. (2018)

Species name	Abbrev.	Origin of isolate	Lifestyle	Size (Mb)	Complete* (%)	Reference
Raffaelea quercivora	RQUE	Discolored sapwood of dead *Quercus mongolica*	Arthropod symbiont	25,9	99,6	Masuya et al. (2016)
Raffaelea quercus-mongolicae	RQMO	*Platypus koryoensis* infested *Quercus mongolica*	Arthropod symbiont	27	98,0	Jeon et al. (2017)
Raffaelea sulphurea	RSUL	*Xyleborus saxesenii* gallery in *Populus deltoides*	Arthropod symbiont	23,2	98,5	Vanderpool et al. (2018)
Sodiomyces alkalinus	SALK	Alkaline soil	Saprophyte/Alkaliphile	43,1	98,7	Grum-Grzhimaylo *et al*. (2018)
Sporothrix brasiliensis	SBRA	Feline skin lesion	Animal pathogen	33,2	98,7	Teixeira et al. (2014)
Sporothrix globosa	SGLO	Face lesion	Animal pathogen	33,5	98,6	Huang et al. (2016)
Stanjemonium grisellum	SGRI	Soil *Artemisia tridentata* grassland	Saprophyte/Marine	31,9	98,9	JGI
Stagonospora nodorum	SNOD	Wheat	Plant pathogen	37,1	97,4	Syme *et al*. (2013)
Sporothrix pallida	SPAL	Soil	Saprophyte	37,8	91,3	D'Alessandro et al. (2016)
Sporothrix schenckii	SSCH	Patient manifesting subcutaneous sporotrichosis	Animal pathogen	32,4	98,5	Teixeira et al. (2014)
Thielavia antarctica	TANT	*Usnea* cf. *aurantio-atra*	Saprophyte/Thermophile	39,4	98,0	JGI
Thielavia appendiculata	TAPP	Dung of rabbit	Saprophyte/Mesophile	32,2	99,1	JGI
Thielavia arenaria	TARE	Desert soil	Saprophyte/ Thermophile	30,7	98,9	JGI
Trichoderma asperellum	TASP	*Sclerotinia minor* sclerotium buried in soil	Mycoparasite	37,4	99,3	Druzhinina et al. (2018)
Trichoderma atroviridae	TATR	Soil	Mycoparasite	36,1	99,4	Kubicek et al. (2011)
Thielaviopsis ethacetica	TETH	Sugar cane	Plant pathogen	29,3	98,1	JCM-Riken BioResource Center, 2018a
Thielaviopsis euricoi	TEUR	Stem of *Metroxylon sagus*	Plant pathogen	29,6	98,0	JCM-Riken BioResource Center, 2018b
Trichoderma harzianum	THAR	Soil	Mycoparasite	41	99,1	Druzhinina et al. (2018)
Thielavia hyrcaniae	THYR	Dung of gazelle	Saprophyte/ Mesophile	20,2	99,2	JGI
Trichoderma longibrachiatum	TLON	Mud	Saprophyte	31,2	89,2	Druzhinina et al. (2018)
Thielaviopsis musarum	TMUS	*Musa* sp.	Plant pathogen	28,3	96,9	Wingfield et al. (2015b)
Thielaviopsis punctulata	TPUN	Phoenix dactylifers	Plant pathogen	27,6	97,2	Wingfield et al. (2015a)
Trichoderma reesei	TREE	Canvas shelter	Mesophile/Saprophyte	33,4	99,3	Martinez et al. (2008)
Thielavia terrestris	TTER	Soil	Saprophyte/Thermophile	36,9	98,5	Berka *et al*. (2011)
Trichoderma virens	TVIR	Soil cultivated with cotton	Mycoparasite	39	98,9	Kubicek et al. (2011)

Species name	Abbrev.	Origin of isolate	Lifestyle	Size (Mb)	Complete* (%)	Reference
Verticillium dahliae	VDAH	*Lactuca sativa*	Plant pathogen	32,9	94,5	Klosterman et al. (2011)
Xylomelasma sordida^	XSOR	*Alnus glutinosa rotten wood, alt. 500 m.*	Saprophyte	49,3	98,3	This study

*Determined using the Ascomycota dataset of BUSCO

Figure 1. Estimated phylogeny and divergence times of the Sordariomycota and basic genome characteristics for the investigated species. Divergence tree generated for the Sordariomycota using BEAST based on 50 shared, single-copy orthologous genes and the topology of the STAG produced supertree. Red blocks at nodes indicate the average genome size for species included below the node. Nodes with species ancestors potentially adapted to arthropod or bark beetle vectors are indicated at the node. Dashed lines indicate the divergence times of the different arthropod vectored Sordariomycete lineages investigated in this study.

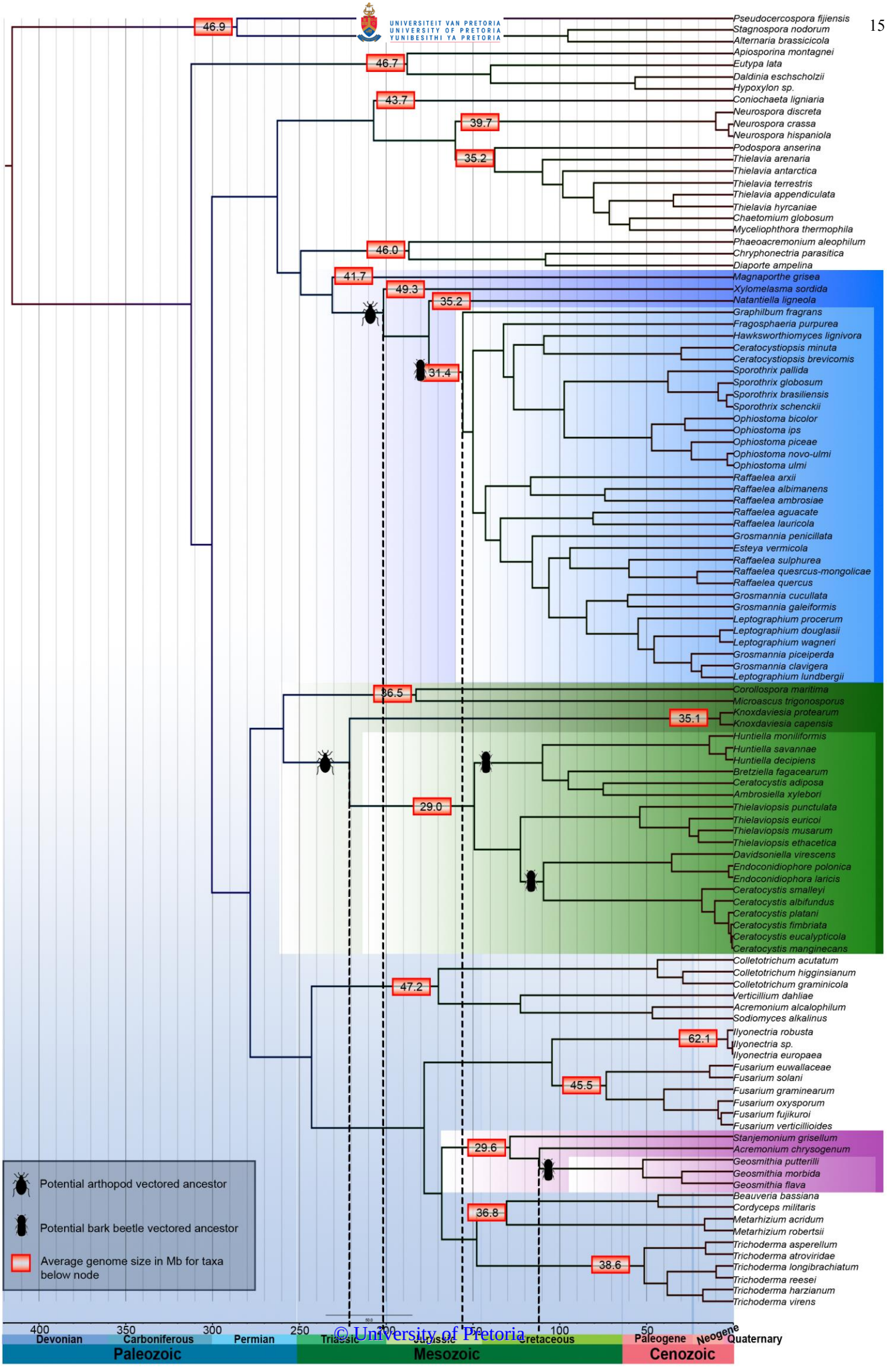
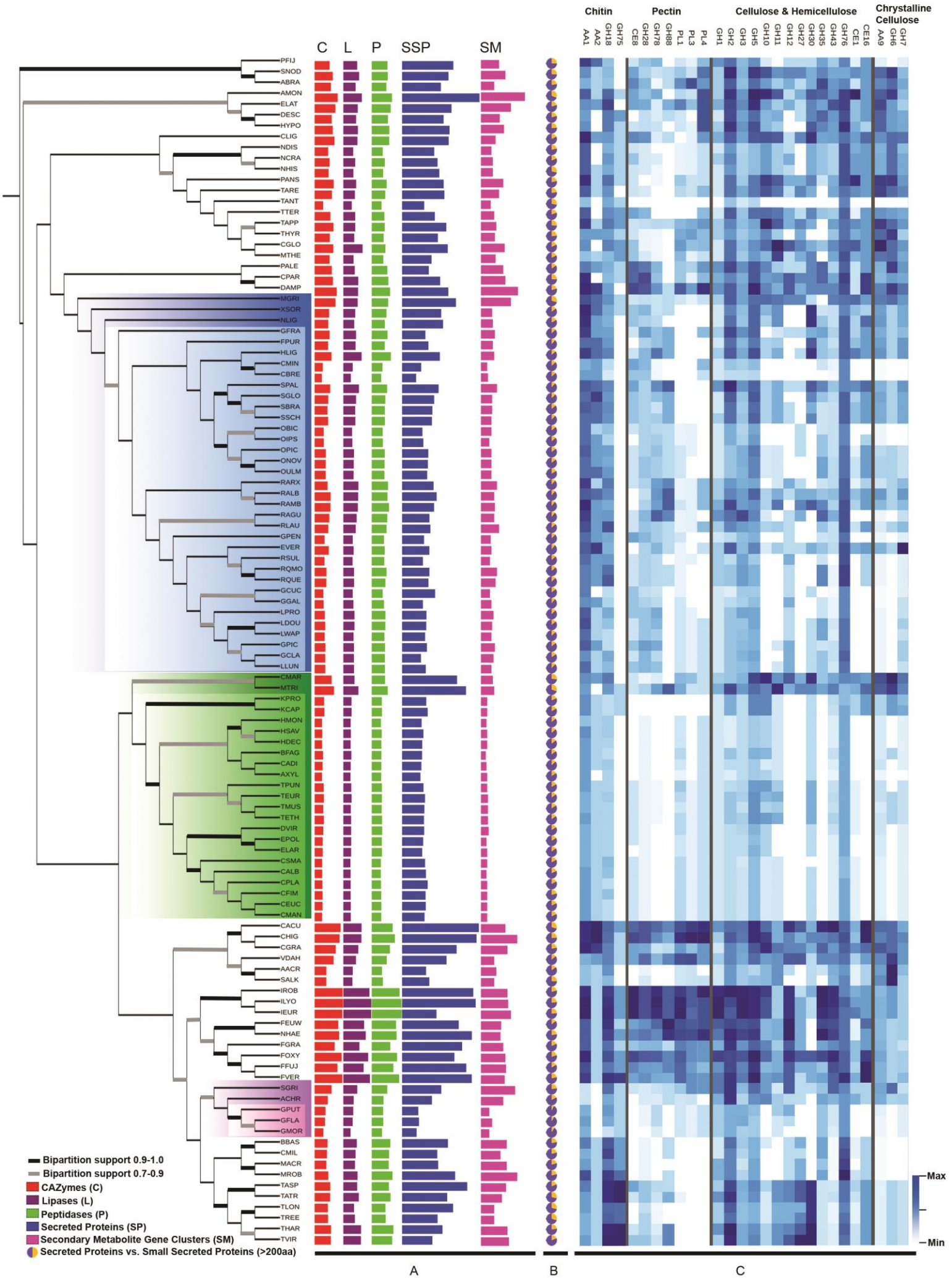


Figure 2. Characteristics for five classes of gene family investigated in this study and the abundance of wood-decay enzymes for the 110 species of Sordariomycota included. (A) Bar graphs representing the number of proteins belonging to each of the different gene families present in the genomes of the different species. (B) The proportion of total secreted proteins made up of small secreted proteins (SSPS; <200aa). The circle represents the total number of secreted proteins (SPs), the yellow portion of the circle indicates the SSPs and the blue indicates the remaining SPs. (C) Heat map showing the abundance of various CAZyme families involved in wood decay in each of the genomes of the investigated species. The darker the blue colour the more representatives of that family are present in the genome of a species.



References

Arango Isaza, R.E., Diaz-Trujillo, C., Dhillon, B., Aerts, A., Carlier, J., et al. (2016). Combating a global threat to a clonal crop: banana black sigatoka pathogen *Pseudocercospora fijiensis* (Synonym *Mycosphaerella fijiensis*) genomes reveal clues for disease control. *PLoS Genetics*, 12, e1005876-e1005876.
DOI: https://doi.org/10.1371/journal.pgen.1005876

Arbuckle, K. & Speed, M.P. (2016). Analysing convergent evolution: A practical guide to methods. In: Pontarotti, P. (Eds.) *Evolutionary Biology*, Springer, pp. 23-36
DOI: https://doi.org/10.1007/978-3-319-41324-2_2

Archie, J.W.A. (1989). A randomization test for phylogenetic information in systematic data. *Systematic Zoology*, 38, 239-252.
DOI: https://doi.org/10.2307/2992285

Aylward, J., Steenkamp, E.T., Dreyer, L.L., Roets, F., Wingfield, B.D., & Wingfield, M.J. (2016). Genome sequences of *Knoxdaviesia capensis* and *K. proteae* (Fungi: Ascomycota) from *Protea* trees in South Africa. *Standards in Genomic Sciences*, 11, 22.
DOI: https://doi.org/10.1186/s40793-016-0139-9

Beimforde, C., Feldberg, K., Nylinder, S., Rikkinen, J., Tuovila, H., et al. (2014). Estimating the Phanerozoic history of the Ascomycota lineages: Combining fossil and molecular data. *Molecular Phylogenetics and Evolution*, 78, 386-398.
DOI: https://doi.org/10.1016/j.ympev.2014.04.024

Berka, R.M., Grigoriev, I.V., Otillar, R., Salamov, A., Grimwood, J., et al. (2011). Comparative genomic analysis of the thermophilic biomass-degrading fungi *Myceliophthora thermophila* and *Thielavia terrestris*. *Nature Biotechnology*, 29, 922-927.
DOI: https://doi.org/10.1038/nbt.1976

Bills, G.F., & Gloer, J.B. (2016). Biologically active secondary metabolites from the fungi. *Microbiology Spectrum*, 4, 1087-1119.
DOI: https://doi.org/10.1128/microbiolspec.FUNK-0009-2016

Blanco-Ulate, B., Rolshausen, P.E., & Cantu, D. (2013a). Draft genome sequence of the grapevine dieback fungus *Eutypa lata* UCR-EL1. *Genome Announcements*, 1, e00228-00213.
DOI: https://doi.org/10.1128/genomeA.00228-13

Blanco-Ulate, B., Rolshausen, P., & Cantu, D. (2013b). Draft genome sequence of the Ascomycete *Phaeoacremonium aleophilum* strain UCR-PA7, a causal agent of the esca disease complex in grapevines. *Genome Announcements*, 1, e00390-00313.
DOI: https://doi.org.10.1128/genomeA.00390-13

Blin, K., Wolf, T., Chevrette, M.G., Lu, X., Schwalen, C.J., et al. (2017). antiSMASH 4.0 — improvements in chemistry prediction and gene cluster boundary identification. *Nucleic Acids Research*, 45, W36-W41.
DOI: https://doi.org/10.1093/nar/gkx319

Bouckaert, R., Vaughan, T.G., Barido-Sottani, J., Duchêne, S., Fourment, M., et al. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, 15, e1006650.
DOI: https://doi.org/10.1371/journal.pcbi.1006650

Brakhage, A.A. (2012). Regulation of fungal secondary metabolism. *Nature Reviews Microbiology*, 11, 21-32.
DOI: https://doi.org/10.1038/nrmicro2916

Caballero, J.R.I., Jeon, J., Lee, Y.H., Fraedrich, S., Klopfenstein, N.B., et al. (2019). Genomic comparisons of the laurel wilt pathogen, *Raffaelea lauricola*, and related tree pathogens highlight an arsenal of pathogenicity related genes. *Fungal Genetics and Biology*, 125, 84-92.
DOI: https://doi.org/10.1016/j.fgb.2019.01.012

Cantarel, B.L., Korf, I., Robb, S.M., Parra, G., Ross, E., et al. (2008). MAKER: An easy-to-use annotation pipeline designed for emerging model organism genomes. *Genome Research*, 18, 188–196.
DOI: https://doi.org/10.1101/gr.6743907

Cantarel, B.L., Coutinho, P.M., Rancurel, C., Bernard, T., Lombard, V. & Henrissat, B. (2008a). The Carbohydrate-Active enZymes database (CAZy): An expert resource for glycogenomics. *Nucleic Acids Research*, 37, D233-D238.
DOI: https://doi.org/10.1093/nar/gkn663

Capella-Gutiérrez, S., Silla-Martínez, J.M. & Gabaldón, T. (2009). trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, 25, 1972–1973.
DOI: https://doi.org/10.1093/bioinformatics/btp348

Carrillo, D., Duncan, R.E., Ploetz, J.N., Campbell, A.F., Ploetz, R.C. & Peña, J.E. (2014). Lateral transfer of a phytopathogenic symbiont among native and exotic ambrosia beetles. *Plant Pathology*, 63, 54-62.
DOI: https://doi.org/10.1111/ppa.12073

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, 17, 540-552.
DOI: https://doi.org/10.1093/oxfordjournals.molbev.a026334

Coleman, J.J., Rounsley, S.D., Rodriguez-Carres, M., Kuo, A., Wasmann, C.C., et al. (2009). The genome of *Nectria haematococca*: Contribution of supernumerary chromosomes to gene expansion. *PLoS Genetics*, 5, e1000618.
DOI: https://doi.org/10.1371/journal.pgen.1000618

Cuomo, C.A., Güldener, U., Xu, J-R., Trail, F., Turgeon, B.G., et al. (2007). The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization. *Science*, 317, 1400-1402.
DOI: https://doi.org/10.1126/science.1143708

Cuomo, C.A., Untereiner, W.A., Ma, L-J., Grabherr, M., & Birren, B.W. (2015). Draft genome sequence of the cellulolytic fungus *Chaetomium globosum*. *Genome Announcements*, 3, e00021-00015.
DOI: https://doi.org/10.1128/genomeA.00021-15

D'Alessandro, E., Giosa, D., Huang, L., Zhang, J., Gao, W., et al. (2016). Draft genome sequence of the dimorphic fungus *Sporothrix pallida*, a nonpathogenic species belonging to *Sporothrix* a genus containing agents of human and feline sporotrichosis. *Genome Announcements*, 4, e00184-00116.
DOI: https://doi.org/10.1128/genomeA.00184-16

Da Silva, R.R. (2017). Bacterial and fungal proteolytic enzymes: Production, catalysis and potential applications. *Applied Biochemistry and Biotechnology*, 183, 1-19.
DOI: https://doi.org/10.1007/s12010-017-2427-2

Dean, R.A., Talbot, N.J., Ebbole, D.J., Farman, M.L., Mitchell, T.K., et al. (2005). The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature*, 434, 980-986.
DOI: https://doi.org/10.1038/nature03449

De Bary, A. (1879). *Die Erscheinung der Symbiose*. Verlag von Karl J. Trübner.
DOI: https://doi.org/10.1515/9783111471839-fm

De Beer, Z.W., Seifert, K.A. & Wingfield, M.J. (2013). The Ophiostomatoid fungi: their dual position in the Sordariomycetes. In: Seifert, K.A., De Beer, Z.W. & Wingfield, M.J. (Eds.), *The Ophiostomatoid Fungi: Expanding Frontiers*, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, pp. 12, 1-19.

De Beer, Z.W. & Wingfield, M.J. (2013). Emerging lineages in the Ophiostomatales. In: Seifert, K.A., De Beer, Z.W. & Wingfield, M.J. (Eds) *The Ophiostomatoid Fungi: Expanding Frontiers*, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, pp. 21-46.

DiGuistini, S., Wang, Y., Liao, N.Y., Taylor, G., Tanguay, P., et al. (2011). Genome and transcriptome analyses of the mountain pine beetle-fungal symbiont *Grosmannia clavigera*, a lodgepole pine pathogen. *Proceedings of the National Academy of Sciences*, 108, 2504-2509.
DOI: https://doi.org/10.1073/pnas.1011289108

Dreaden, T.J., Davis, J.M., De Beer, Z.W., Ploetz, R.C., Soltis, P.S., et al. (2014). Phylogeny of ambrosia beetle symbionts in the genus *Raffaelea*. *Fungal Biology*, 118, 970-978.
DOI: https://doi.org/10.1016/j.funbio.2014.09.001

Druzhinina, I.S., Chenthamara, K., Zhang, J., Atanasova, L., Yang, D., et al. (2018). Massive lateral transfer of genes encoding plant cell wall-degrading enzymes to the mycoparasitic fungus *Trichoderma* from its plant-associated hosts. *PLoS Genetics*, 14, e1007322.
DOI: https://doi.org/10.1371/journal.pgen.1007322

Emanuelsson, O., Nielsen, H., Brunak, S. & Von Heijne, G. (2000). Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. *Journal of Molecular Biology*, 300, 1005-1016.
DOI: https://doi.org/10.1006/jmbi.2000.3903

Emms, D.M., & Kelly, S. (2018a). OrthoFinder2: Fast and accurate phylogenomic orthology analysis from gene sequences. *BioRxiv*, 466201.
DOI: https://doi.org/10.1101/466201

Emms, D.M., & Kelly, S. (2018b). STAG: Species tree inference from all genes. *BioRxiv*, 267914.
DOI: https://doi.org/10.1101/267914

Emms, D.M., & Kelly, S. (2019). OrthoFinder: Phylogenetic orthology inference for comparative genomics. *Genome Biology*, 20, 1-14.
DOI: https://doi.org/10.1186/s13059-019-1832-y

Eskalen, A., Stouthamer, R., Lynch, S.C., Rugman-Jones, P.F., Twizeyimana, M., et al. (2013). Host range of *Fusarium* dieback and its ambrosia beetle (Coleoptera: Scolytinae) vector in Southern California. *Plant Disease*, 97, 938-951.
DOI: https://doi.org/10.1094/PDIS-11-12-1026-RE

Espagne, E., Lespinet, O., Malagnac, F., Da Silva, C., Jaillon, O., Porcel, et al. (2008). The genome sequence of the model ascomycete fungus *Podospora anserina*. *Genome Biology*, 9, R77.
DOI: https://doi.org/10.1186/gb-2008-9-5-r77

Farrell, B.D., Sequeira, A.S., O'Meara, B.C., Normark, B.B., Chung, J.H. & Jordal, B.H. (2001). The evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodinae). *Evolution*, 55, 2011-2027.
DOI: https://doi.org/10.1111/j.0014-3820.2001.tb01318.x

Flutre, T., Duprat, E., Feuillet, C. & Quesneville, H. (2011). Considering transposable element diversification in *de novo* annotation approaches. *PLoS ONE*, 6, e16526.
DOI: https://doi.org/10.1371/journal.pone.0016526

Forgetta, V., Leveque, G., Dias, J., Grove, D., Lyons, R., Jr., et al. (2013). Sequencing of the Dutch elm disease fungus genome using the Roche/454 GS-FLX Titanium System in a comparison of multiple genomics core facilities. *Journal of Biomolecular Techniques*, 24, 39-49.
DOI: https://doi.org/10.7171/jbt.12-2401-005

Frank, A.B. (1877). Uber die biologischen verhaltnisse des thallus eineger krustenflechten. *Beitrage zur Biologie der Pflanzen*, 2S, 123-200.

Freeman, S., Sharon, M., Maymon, M., Mendel, Z., Protasov, A., et al. (2013). *Fusarium euwallaceae* sp. nov. - a symbiotic fungus of *Euwallacea* sp., an invasive ambrosia beetle in Israel and California. *Mycologia*, 105, 1595-1606.
DOI: https://doi.org/10.3852/13-066

Galagan, J.E., Calvo, S.E., Borkovich, K.A., Selker, E.U., Read, N.D., et al. (2003). The genome sequence of the filamentous fungus *Neurospora crassa*. *Nature*, 422, 859-868.
DOI: https://doi.org/10.1038/nature01554

Gao, Q., Jin, K., Ying, S-H., Zhang, Y., Xiao, G., et al. (2011). Genome sequencing and comparative transcriptomics of the model entomopathogenic fungi *Metarhizium anisopliae* and *M. acridum*. *PLoS Genetics*, 7, e1001264.
DOI: https://doi.org/10.1371/journal.pgen.1001264

Grum-Grzhimaylo, A.A., Falkoski, D.L., van den Heuvel, J., Valero-Jiménez, C.A., Min, B., et al. (2018). The obligate alkalophilic soda-lake fungus *Sodiomyces alkalinus* has shifted to a protein diet. *Molecular Ecology*, 27, 4808-4819.
DOI: https://doi.org/10.1111/mec.14912

Hall, B.K. (2013). Homology, homoplasy, novelty, and behaviour. *Developmental Psychobiology*, 55, 4-12.
DOI: https://doi.org/10.1002/dev.21039

Han, J.H., Chon, J.K., Ahn, J.H., Choi, I.Y., Lee, Y.H., & Kim, K.S. (2016). Whole genome sequence and genome annotation of *Colletotrichum acutatum*, causal agent of anthracnose in pepper plants in South Korea. *Genomics Data*, 8, 45-46.
DOI: https://doi.org/10.1016/j.gdata.2016.03.007

Haridas, S., Wang, Y., Lim, L., Massoumi Alamouti, S., Jackman, S., et al. (2013). The genome and transcriptome of the pine saprophyte *Ophiostoma piceae*, and a comparison with the bark beetle-associated pine pathogen *Grosmannia clavigera*. *BMC Genomics*, 14, 373.
DOI: https://doi.org/10.1186/1471-2164-14-373

Heath, R.N., Wingfield, M.J., Wingfield, B.D., Meke, G., Mbaga, A. & Roux, J. (2009a). *Ceratocystis* species on *Acacia mearnsii* and *Eucalyptus* spp. in Eastern and Southern Africa including six new species. *Fungal Diversity*, 34, 41-67.

Heath, R.N., Wingfield, M.J., Van Wyk, M. & Roux, J. (2009b). Insect associates of *Ceratocystis albifundus* and patterns of association in a native savanna ecosystem in South Africa. *Environmental Entomology*, 38, 356-364.
DOI: https://doi.org/10.1603/022.038.0207

Hoffmeister, D. & Keller, N.P. (2007). Natural products of filamentous fungi: Enzymes, genes, and their regulation. *Natural Product Reports*, 24, 393-416.
DOI: https://doi.org/10.1039/B603084J

Hofstetter, R.W., Moser, J. & Blomquist, S. (2013). Mites associated with bark beetles and their hyperphoretic Ophiostomatoid fungi. In: Seifert, K.A., De Beer, Z.W. & Wingfield, M.J. (Eds.) *The Ophiostomatoid Fungi: Expanding Frontiers*, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, pp. 165-176.

Hsiau, P. T.W. & Harrington, T.C. (1997). *Ceratocystiopsis brevicomi* sp. nov., a mycangial fungus from *Dendroctonus brevicomis* (Coleoptera: Scolytidae). *Mycologia*, 89, 661-669.
DOI: https://doi.org/10.1080/00275514.1997.12026831

Huang, L., Gao, W., Giosa, D., Criseo, G., Zhang, J., et al. (2016). Whole-genome sequencing and *in silico* analysis of two strains of *Sporothrix globosa*. *Genome Biology and Evolution*, 8, 3292-3296.

DOI: https://doi.org/10.1093/gbe/evw230

Hulcr, J. & Stelinski, L.L. (2017). The ambrosia symbiosis: From evolutionary ecology to practical management. *Annual Review of Entomology*, 62, 285-303.
DOI: https://doi.org/10.1146/annurev-ento-031616-035105

Ibarra-Laclette, E., Sánchez-Rangel, D., Hernández-Domínguez, E., Pérez-Torres, C.A., Ortiz-Castro, R., et al. (2017). Draft genome sequence of the phytopathogenic fungus *Fusarium euwallaceae*, the causal agent of *Fusarium* dieback. *Genome Announcements*, 5, e00881-00817.
DOI: https://doi.org/10.1128/genomeA.00881-17

JCM-Riken BioResource Center (2018a). NBRP: Genome sequencing of eukaryotic microorganisms (*Thielaviopsis ethacetica* JCM 6961).
Retrieved from https://www.ncbi.nlm.nih.gov/nuccore/BCFY00000000.1

JCM-Riken BioResource Center (2108b). NBRP: Genome sequencing of eukaryotic microorganisms (*Thielaviopsis euricoi* JCM6020).
Retrieved from https://www.ncbi.nlm.nih.gov/nuccore/BCHJ00000000.1

Jeon, J., Kim, K.T., Song, H., Lee, G.W., Cheong, K., et al. (2017). Draft genome sequence of the fungus associated with oak wilt mortality in South Korea *Raffaelea quercus-mongolicae* KACC44405. *Genome Announcements*, 5, e00797-00717.
DOI: https://doi.org/doi:10.1128/genomeA.00797-17

Jiménez, D.J., Hector, R.E., Riley, R., Lipzen, A., Kuo, R.C., et al. (2017). Draft genome sequence of *Coniochaeta ligniaria* NRRL 30616, a lignocellulolytic fungus for bioabatement of inhibitors in plant biomass hydrolysates. *Genome Announcements*, 5, e01476-01416.
DOI: https://doi.org/10.1128/genomeA.01476-16

Kasson, M.T., O'Donnell, K., Rooney, A.P., Sink, S., Ploetz, R.C., et al. (2013). An inordinate fondness for *Fusarium*: phylogenetic diversity of fusaria cultivated by ambrosia beetles in the genus *Euwallacea* on avocado and other plant hosts. *Fungal Genetics and Biology*, 56, 147-157.
DOI: https://doi.org/10.1016/j.fgb.2013.04.004

Keller, N.P., Turner, G. & Bennett, J.W. (2005). Fungal secondary metabolism—from biochemistry to genomics. *Nature Reviews Microbiology*, 3, 937-947.
DOI: https://doi.org/10.1038/nrmicro1286

Kjærbølling, I., Vesth, T.C., Frisvad, J.C., Nybo, J.L., Theobald, S., et al. (2018). Linking secondary metabolites to gene clusters through genome sequencing of six diverse *Aspergillus* species. *Proceedings of the National Academy of Sciences*, 115, E753-E761.
DOI: https://doi.org/10.1073/pnas.1715954115

Khoshraftar, S., Hung, S., Khan, S., Gong, Y., Tyagi, V., et al. (2013). Sequencing and annotation of the *Ophiostoma ulmi* genome. *BMC Genomics*, 14, 162.
DOI: https://doi.org/10.1186/1471-2164-14-162

Klosterman, S.J., Subbarao, K.V., Kang, S., Veronese, P., Gold, S.E., et al. (2011). Comparative genomics yields insights into niche adaptation of plant vascular wilt pathogens. *PLoS Pathogens*, 7, e1002137.
DOI: https://doi.org/10.1371/journal.ppat.1002137

Kolařík, M. & Kirkendall, L.R. (2010). Evidence for a new lineage of primary ambrosia fungi in *Geosmithia* Pitt (Ascomycota: Hypocreales). *Fungal Biology*, 114, 676-689.
DOI: https://doi.org/10.1016/j.funbio.2010.06.005

Kolařík, M. & Jankowiak, R. (2013). Vector affinity and diversity of *Geosmithia* fungi living on subcortical insects inhabiting Pinaceae species in Central and Northeastern Europe. *Microbial Ecology*, 66, 682-700.
DOI: https://doi.org/10.1007/s00248-013-0228-x

Krogh, A., Larsson, B., Von Heijne, G. & Sonnhammer, E.L. (2001). Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. *Journal of Molecular Biology*, 305, 567-580.
DOI: https://doi.org/10.1006/jmbi.2000.4315

Kubicek, C.P., Herrera-Estrella, A., Seidl-Seiboth, V., Martinez, D.A., Druzhinina, I.S., et al. (2011). Comparative genome sequence analysis underscores mycoparasitism as the ancestral lifestyle of *Trichoderma*. *Genome Biology*, 12, R40.
DOI: https://doi.org/10.1186/gb-2011-12-4-r40

Kück, P. & Meusemann, K. (2010). FASconCAT: Conveniet handling of data matrices. *Molecular Phylogenetics and Evolution*, 56, 1115-1118.
DOI: https://doi.org/10.1016/j.ympev.2010.04.024

Lah, L., Löber, U., Hsiang, T. & Hartmann, S. (2017). A genomic comparison of putative pathogenicity-related gene families in five members of the Ophiostomatales with different lifestyles. *Fungal Biology*, 121, 234–252.
DOI: https://doi.org/10.1016/j.funbio.2016.12.002

Lanfear, R., Calcott, B., Kainer, D., Mayer, C. & Stamatakis, A. (2014). Selecting optimal partitioning schemes for phylogenomic datasets. *BMC Evolutionary Biology*,14, 82.
DOI: https://doi.org/10.1186/1471-2148-14-82

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T. & Calcott, B. (2017). PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, 34, 772–773.
DOI: https://doi.org/10.1093/molbev/msw260

Lawrence, J.G. (2005). Common themes in the genome strategies of pathogens. *Current Opinion in Genetics & Development*, 15, 584-588.
DOI: https://doi.org/10.1016/j.gde.2005.09.007

Lind, A.L., Wisecaver, J.H., Lameiras, C., Wiemann, P., Palmer, J.M., et al. (2017). Drivers of genetic diversity in secondary metabolic gene clusters within a fungal species. *PLoS Biology*, 15, e2003583.
DOI: https://doi.org/10.1371/journal.pbio.2003583

Lomsadze, A., Ter-Hovhannisyan, V., Chernoff, Y.O. & Borodovsky, M. (2005). Gene identification in novel eukaryotic genomes by self-training algorithm. *Nucleic Acids Research*, 33, 6494–6506.
DOI: https://doi.org/10.1093/nar/gki937

Löytynoja, A. (2014). Phylogeny-aware alignment with PRANK. In: Russel, D. (Eds.), *Multiple Sequence Alignment Methods*, Springer, pp. 155-170.
DOI: https://doi.org/10.1007/978-1-62703-646-7_10

Lynn, K.M.T., Wingfield, M.J., Durán. A., Marincowitz, S., Oliveira, L.S.S., et al. (2020). *Euwallacea perbrevis* (Coleoptera: Curculionidae: Scolytinae), a confirmed pest on *Acacia crassicarpa* in Riau, Indonesia, and a new fungal symbiont; *Fusarium rekanum* sp. nov. *Antonie van Leeuwenhoek*, 113, 803-823.
DOI: https://doi.org/10.1007/s10482-020-01392-8

Ma, L.J., Van Der Does, H.C., Borkovich, K.A., Coleman, J.J., Daboussi, M-J., et al. (2010). Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature*, 464, 367.
DOI: https://doi.org/10.1038/nature08850

Maharachchikumbura, S.S., Hyde, K.D., Jones, E.G., McKenzie, E.H.C., Bhat, J.D., et al. (2016). Families of Sordariomycetes. *Fungal Diversity*, 79, 1-317.
DOI: https://doi.org/10.1007/s13225-016-0369-6

Martin, B.D. & Schwab, E. (2012). Symbiosis: "Living together" in chaos. *Studies in the History of Biology*, 4, 7-25.

Martinez, D., Berka, R. M., Henrissat, B., Saloheimo, M., Arvas, M., et al. (2008). Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*). *Nature Biotechnology*, 26, 553-560.
DOI: https://doi.org/10.1038/nbt1403

Masuya, H., Manabe, R.I., Ohkuma, M., & Endoh, R. (2016). Draft genome sequence of *Raffaelea quercivora* JCM 11526, a Japanese oak wilt pathogen associated with the platypodid beetle, *Platypus quercivorus*. *Genome Announcements*, 4, e00755-00716.
DOI: https://doi.org/10.1128/genomeA.00755-16

McCutcheon, J.P. & Moran, N.A. (2012). Extreme genome reduction in symbiotic bacteria. *Nature Reviews Microbiology*, 10, 13-26.
DOI: https://doi.org/10.1038/nrmicro2670

Morales-Cruz, A., Amrine, K.C.H., Blanco-Ulate, B., Lawrence, D.P., Travadon, R., et al. (2015). Distinctive expansion of gene families associated with plant cell wall degradation, secondary metabolism, and nutrient uptake in the genomes of grapevine trunk pathogens. *BMC Genomics*, 16, 469.
DOI: https://doi.org/10.1186/s12864-015-1624-z

Moser, J.C., Perry, T.J., Bridges, J.R. & Yin, H.F. (1995). Ascospore dispersal of *Ceratocystiopsis ranaculosus*, a mycangial fungus of the southern pine beetle. *Mycologia*, 87, 84-86.
DOI: https://doi.org/10.1080/00275514.1995.12026506

Mueller, U.G., Gerardo, N.M., Aanen, D.K., Six, D.L. & Schultz, T.R. (2005). The evolution of agriculture in insects. *Annual Review of Ecology, Evolution, and Systematics*, 36, 563-595.
DOI: https://doi.org/10.1146/annurev.ecolsys.36.102003.152626

Mushegian, A. (1999). The minimal genome concept. *Current Opinion in Genetics & Development*, 9, 709-714.
DOI: https://doi.org/10.1016/S0959-437X(99)00023-4

Nguyen, L.T., Schmidt, H.A., Von Haeseler, A. & Minh, B.Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, 32, 268-274.
DOI: https://doi.org/10.1093/molbev/msu300

O'Connell, R.J., Thon, M.R., Hacquard, S., Amyotte, S.G., Kleemann, J., et al. (2012). Lifestyle transitions in plant pathogenic *Colletotrichum* fungi deciphered by genome and transcriptome analyses. *Nature Genetics*, 44, 1060-1065.
DOI: https://doi.org/10.1038/ng.2372

Ohm, R.A., Feau, N., Henrissat, B., Schoch, C.L., Horwitz, B.A., *et al*. (2012). Diverse lifestyles and strategies of plant pathogenesis encoded in the genomes of eighteen Dothideomycete fungi. *PLoS Pathogens*, 8, e1003037.
DOI: https://doi.org/10.1371/journal.ppat.1003037

Petersen, T.N., Brunak, S., von Heijne, G. & Nielsen, H. (2011). SignalP 4.0: Discriminating signal peptides from transmembrane regions. *Nature Methods*, 8, 785-786.
DOI: https://doi.org/10.1038/nmeth.1701

Pérez-Ortega, S., Garrido-Benavent, I., Grube, M., Olmo, R. & De Los Ríos, A. (2016). Hidden diversity of marine borderline lichens and a new order of fungi: Collemopsidiales (Dothideomyceta). *Fungal Diversity*, 80, 285-300.
DOI: https://doi.org/10.1007/s13225-016-0361-1

Ploetz, R.C., Hulcr, J., Wingfield, M.J. & De Beer, Z.W. (2013). Destructive tree diseases associated with ambrosia and bark beetles: Black swan events in tree pathology? *Plant Disease*, 97, 856-872.
DOI: https://doi.org/10.1094/PDIS-01-13-0056-FE

Quesneville, H., Bergman, C.M., Andrieu, O., Autard, D., Nouaud, D., et al. (2005). Combined evidence annotation of transposable elements in genome sequences. *PLoS Computational Biology*, 1, e22.
DOI: https://doi.org/10.1371/journal.pcbi.0010022

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, 67, 901-904.
DOI: https://doi.org/10.1093/sysbio/syy032

Rawlings, N.D., Barrett, A.J., Thomas, P.D., Huang, X., Bateman, A. and Finn, R.D. (2017). The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database. *Nucleic Acids Research*, 46, D624-D632.
DOI: https://doi.org/10.1093/nar/gkx1134

Réblová, M. (2006). Molecular systematics of *Ceratostomella* sensu lato and morphologically similar fungi. *Mycologia*, 98, 68-93.
DOI: https://doi.org/10.1080/15572536.2006.11832714

Réblová, M. & Štěpánek, V. (2009). New fungal genera, *Tectonidula* gen. nov. for *Calosphaeria*-like fungi with holoblastic-denticulate conidiogenesis and *Natantiella* gen. nov. for three species segregated from *Ceratostomella*. *Mycological Research*, 113, 991-1002.
DOI: https://doi.org/10.1016/j.mycres.2009.06.003

Rep, M. (2005). Small proteins of plant-pathogenic fungi secreted during host colonization. *FEMS Microbiology Letters*, 253, 19-27.
DOI: https://doi.org/10.1016/j.femsle.2005.09.014

Riley, R., Salamov, A.A., Brown, D.W., Nagy, L.G., Floudas, D., (2014). Extensive sampling of basidiomycete genomes demonstrates inadequacy of the white-rot/brown-rot paradigm for wood decay fungi. *Proceedings of the National Academy of Sciences*, 111, 9923-9928.
DOI: https://doi.org/10.1073/pnas.1400592111

Saucedo-Carabez, J.R., Ploetz, R.C., Konkol, J.L., Carrillo, D. & Gazis, R. (2018). Partnerships between ambrosia beetles and fungi: Lineage-specific promiscuity among vectors of the laurel wilt pathogen, *Raffaelea lauricola*. *Microbial Ecology*, 76, 925-940.
DOI: https://doi.org/10.1007/s00248-018-1188-y

Schuelke, T.A., Wu, G., Westbrook, A., Woeste, K., Plachetzki, D.C., et al. (2017). Comparative genomics of pathogenic and nonpathogenic beetle-vectored fungi in the genus *Geosmithia*. *Genome Biology and Evolution*, 9, 3312-3327.
DOI: https://doi.org/10.1093/gbe/evx242

Shen X-X, Hittinger CT, Rokas A. 2017. Contentious relationships in phylogenomic studies can be driven by a handful of genes. *Nature Ecology & Evolution* 1: 0126.
DOI: https://doi.org/10.1038/s41559-017-0126.

Simão, F.A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E.V. & Zdobnov, E.M. (2015). BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*, 31, 3210-3212.
DOI: https://doi.org/10.1093/bioinformatics/btv351

Six, D.L. (2012). Ecological and evolutionary determinants of bark beetle—fungus symbioses. *Insects*, 3, 339-366.
DOI: https://doi.org/10.3390/insects3010339

Six DL (2019). A major symbiont shift supports a major niche shift in a clade of tree-killing bark beetles. *Ecological Entomology*, 45, 190-201.
DOI: https://doi.org/10.1111/een.12786

Skelton, J., Jusino, M.A., Carlson, P.M., Smith, K., Banik, M.T., et al. (2019). Relationships among wood-boring beetles, fungi, and the decomposition of forest biomass. *Molecular Ecology*, 28, 4971-4986.
DOI: https://doi.org/10.1111/mec.15263

Souza, P.M.D., Bittencourt, M.L.D.A., Caprara, C.C., Freitas, M.D., Almeida, R.P.C.D., et al. (2015). A biotechnology perspective of fungal proteases. *Brazilian Journal of Microbiology*, 46, 337-346.
DOI: http://dx.doi.org/10.1590/S1517-838246220140359

Spatafora, J.W. & Blackwell, M. (1994). The polyphyletic origins of Ophiostomatoid fungi. *Mycological Research*, 98, 1-9.
DOI: https://doi.org/10.1016/S0953-7562(09)80327-4

Spatafora, J.W., Sung, G.H., Johnson, D., Hesse, C., O'Rourke, B., et al. (2006). A five-gene phylogeny of Pezizomycotina. *Mycologia*, 98, 1018-1028.
DOI: https://doi.org/10.1080/15572536.2006.11832630

Stamatakis, A. (2006). RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22, 2688–2690.
DOI: https://doi.org/10.1093/bioinformatics/btl446

Stanke, M., Keller, O., Gunduz, I., Hayes, A., Waack, S. & Morgenstern, B. (2006). AUGUSTUS: *Ab initio* prediction of alternative transcripts. *Nucleic Acids Research*, 34, W435–W439.
DOI: https://doi.org/10.1093/nar/gkl200

Swofford, D. L. (1998) *Phylogenetic analysis using parsimony*, The Smithsonian Institution.

Syme, R.A., Hane, J.K., Friesen, T.L., & Oliver, R.P. (2013). Resequencing and comparative genomics of *Stagonospora nodorum*: Sectional gene absence and effector discovery. *G3: Genes, Genomes, Genetics*, 3, 959-969.
DOI: https://doi.org/10.1534/g3.112.004994

Terfehr, D., Dahlmann, T.A., Specht, T., Zadra, I., Kürnsteiner, H., & Kück, U. (2014). Genome sequence and annotation of *Acremonium chrysogenum*, producer of the β-lactam antibiotic cephalosporin C. *Genome Announcements*, 2, e00948-00914.
DOI: https://doi.org/10.1128/genomeA.00948-14

Ter-Hovhannisyan, V., Lomsadze, A., Chernoff, Y.O. & Borodovsky, M. (2008). Gene prediction in novel fungal genomes using an *ab initio* algorithm with unsupervised training. *Genome Research*, 18, 1979–1990.
DOI: https://doi.org/10.1101/gr.081612.108

Teixeira, M.M., De Almeida, L.G.P., Kubitschek-Barreira, P., Alves, F.L., Kioshima, É.S. et al. (2014). Comparative genomics of the major fungal agents of human and animal Sporotrichosis: *Sporothrix schenckii* and *Sporothrix brasiliensis*. *BMC Genomics*, 15, 943.
DOI: https://doi.org/10.1186/1471-2164-15-943

Valiev, A., Ogel, Z.B. & Klepzig, K.D. (2009). Analysis of cellulase and polyphenol oxidase production by southern pine beetle associated fungi. *Symbiosis*, 49, 37-42.
DOI: https://doi.org/10.1007/s13199-009-0007-0

Van Der Nest, M.A., Bihon, W., De Vos, L., Naidoo, K., Roodt, D., et al. (2014a). Draft genome sequences of *Diplodia sapinea*, *Ceratocystis manginecans*, and *Ceratocystis moniliformis*. *IMA Fungus*, 5, 135-140.
DOI: https://doi.org/10.5598/imafungus.2014.05.01.13

Van Der Nest, M.A., Beirn, L.A., Crouch, J.A., Demers, J.E., De Beer, Z.W., et al. (2014b). Draft genomes of *Amanita jacksonii*, *Ceratocystis albifundus*, *Fusarium circinatum*, *Huntiella omanensis*, *Leptographium procerum*, *Rutstroemia sydowiana*, and *Sclerotinia echinophila*. *IMA Fungus*, 5, 472-485.
DOI: https://doi.org/10.5598/imafungus.2014.05.02.11

Van Der Nest, M.A., Steenkamp, E.T., McTaggart, A.R., Trollip, C., Godlonton, T., et al. (2015). Saprophytic and pathogenic fungi in the Ceratocystidaceae differ in their ability to metabolize plant-derived sucrose. *BMC Evolutionary Biology*, 15, 273-273.
DOI: https://doi.org/10.1186/s12862-015-0550-7

Vanderpool, D., Bracewell, R.R. & McCutcheon, J.P. (2018). Know your farmer: Ancient origins and multiple independent domestications of ambrosia beetle fungal cultivars. *Molecular Ecology*, 27, 2077-2094.
DOI: https://doi.org/10.1111/mec.14394

Vesth, T.C., Nybo, J.L., Theobald, S., Frisvad, J.C., Larsen, T.O., et al. (2018). Investigation of inter- and intraspecies variation through genome sequencing of *Aspergillus* section *Nigri*. *Nature Genetics*, 50, 1688-1695.
DOI: https://doi.org/10.1038/s41588-018-0246-1

Whiston, E. & Taylor, J.W. (2016). Comparative phylogenomics of pathogenic and nonpathogenic species. *G3: Genes, Genomes, Genetics*, 6, 235-244.
DOI: https://doi.org/10.1534/g3.115.022806

Wiemann, P., Sieber, C.M.K., von Bargen, K.W., Studt, L., Niehaus, E-M., et al. (2013). Deciphering the cryptic genome: Genome-wide analyses of the rice pathogen *Fusarium fujikuroi* reveal complex regulation of secondary metabolism and novel metabolites. *PLoS Pathogens*, 9, e1003475.
DOI: https://doi.org/10.1371/journal.ppat.1003475

Wilken, P.M., Steenkamp, E.T., Wingfield, M.J., De Beer, Z.W., & Wingfield, B.D. (2013). Draft nuclear genome sequence for the plant pathogen, *Ceratocystis fimbriata*. *IMA Fungus*, 4, 357-358.
DOI: https://doi.org/10.5598/imafungus.2013.04.02.14

Wingfield, B.D., Ades, P.K., Al-Naemi, F.A., Beirn, L.A., Bihon, W., et al. (2015a). Draft genome sequences of *Chrysoporthe austroafricana*, *Diplodia scrobiculata*, *Fusarium nygamai*, *Leptographium lundbergii*, *Limonomyces culmigenus*, *Stagonosporopsis tanaceti*, and *Thielaviopsis punctulata*. *IMA Fungus*, 6, 233-248.
DOI: https://doi.org/10.5598/imafungus.2015.06.01.15

Wingfield, B.D., Barnes, I., De Beer, Z.W., De Vos, L., Duong, T.A., et al. (2015b). Draft genome sequences of *Ceratocystis eucalypticola*, *Chrysoporthe cubensis*, *C. deuterocubensis*, *Davidsoniella virescens*, *Fusarium temperatum*, *Graphilbum fragrans*, *Penicillium nordicum*, and *Thielaviopsis musarum*. *IMA Fungus*, 6, 493-506.
DOI: https://doi.org/10.5598/imafungus.2015.06.02.13

Wingfield, B.D., Ambler, J.M., Coetzee, M., De Beer, Z.W., Duong, T.A., et al. (2016a) Draft genome sequences of *Armillaria fuscipes*, *Ceratocystiopsis minuta*, *Ceratocystis adiposa*, *Endoconidiophora laricicola*, *E. polonica* and *Penicillium freii* DAOMC 242723. *IMA Fungus*, 7, 217-227.
DOI: https://doi.org/10.5598/imafungus.2016.07.01.11

Wingfield, B.D., Duong, T.A., Hammerbacher, A., Van Der Nest, M.A., Wilson, A., et al. (2016b) Draft genome sequences for *Ceratocystis fagacearum*, *C. harringtonii*, *Grosmannia penicillata*, *and Huntiella bhutanensis*. *IMA Fungus*, 7, 317-323.
DOI: https://doi.org/10.5598/imafungus.2016.07.02.11

Wingfield, B.D., Berger, D.K., Steenkamp, E.T., Lim, H.J., Duong, T.A., et al. (2017). Draft genome of *Cercospora zeina*, *Fusarium pininemorale*, *Hawksworthiomyces lignivorus*, *Huntiella decipiens* and *Ophiostoma ips*. *IMA Fungus*, 8, 385-396.
DOI: https://doi.org/10.5598/imafungus.2017.08.02.10

Wingfield, B.D., Bills, G.F., Dong, Y., Huang, W., Nel, W.J., et al. (2018a). Draft genome sequence of *Annulohypoxylon stygium*, *Aspergillus mulundensis*, *Berkeleyomyces basicola* (syn. *Thielaviopsis basicola*), *Ceratocystis smalleyi*, two *Cercospora beticola* strains, *Coleophoma cylindrospora*, *Fusarium fracticaudum*, *Phialophora* cf. *hyalina*, and *Morchella septimelata*. *IMA Fungus*, 9, 199-223.
DOI: https://doi.org/10.5598/imafungus.2018.09.01.13

Wingfield, B.D., Liu, M., Nguyen, H.D., Lane, F.A., Morgan, S.W., et al. (2018b). Nine draft genome sequences of *Claviceps purpurea* s. lat., including *C. arundinis*, *C. humidiphila*, and *C.* cf. *spartinae*, pseudomolecules for the pitch canker pathogen *Fusarium circinatum*, draft genome of *Davidsoniella eucalypti*, *Grosmannia galeiformis*, *Quambalaria eucalypti*, and *Teratosphaeria destructans*. *IMA Fungus*, 9, 401-418
DOI: https://doi.org/10.5598/imafungus.2018.09.02.10

Wingfield, M.J., Barnes, I., De Beer, Z.W., Roux, J., Wingfield, B.D. & Taerum, S.J. (2017). Novel associations between Ophiostomatoid fungi, insects, and tree hosts: Current status—future prospects. *Biological Invasions*, 18, 3215–3228.
DOI: https://doi.org/10.1007/s10530-016-1084-7

Wu, W., Davis, R.W., Tran-Gyamfi, M.B., Kuo, A., LaButti, K., et al. (2017). Characterization of four endophytic fungi as potential consolidated bioprocessing hosts for conversion of lignocellulose into advanced biofuels. *Applied Microbiology and Biotechnology*, 101, 2603-2618.
DOI: https://doi.org/10.1007/s00253-017-8091-1

Xiao, G., Ying, S.-H., Zheng, P., Wang, Z.L., Zhang, S., *et al*. (2012). Genomic perspectives on the evolution of fungal entomopathogenicity in *Beauveria bassiana*. *Scientific Reports*, 2, 483.
DOI: https://doi.org/10.1038/srep00483

Zampounis, A., Pigné, S., Dallery, J.F., Wittenberg, A.H.J., Zhou, S., et al. (2016). Genome sequence and annotation of *Colletotrichum higginsianum*, a causal agent of crucifer anthracnose disease. *Genome Announcements*, 4, e00821-00816.
DOI: https://doi.org/10.1128/genomeA.00821-16

Zheng, P., Xia, Y., Xiao, G., Xiong, C., Hu, X., et al. (2011). Genome sequence of the insect pathogenic fungus *Cordyceps militaris*, a valued traditional Chinese medicine. *Genome Biology*, 12, R116.
DOI: https://doi.org/10.1186/gb-2011-12-11-r116

Zhang, N., Castlebury, L.A., Miller, A.N., Huhndorf, S.M., Schoch, C.L., et al. (2006). An overview of the systematics of the Sordariomycetes based on a four-gene phylogeny. *Mycologia*, 98, 1076-1087.
DOI: https://doi.org/10.1080/15572536.2006.11832635

Zhang, H., Yohe, T., Huang, L., Entwistle, S., Wu, P., et al. (2018). dbCAN2: A meta server for automated carbohydrate-active enzyme annotation. *Nucleic Acids Research*, 46, W95-W101.
DOI: https://doi.org/10.1093/nar/gky418

Zipfel, R.D., De Beer, Z.W., Jacobs, K., Wingfield, B.D. & Wingfield, M.J. (2006). Multi-gene phylogenies define *Ceratocystiopsis* and *Grosmannia* distinct from *Ophiostoma*. *Studies in Mycology*, 55, 75-97.
DOI: https://doi.org/10.3114/sim.55.1.75

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Methods

Genome sequencing

Genome sequences of *Natantiella ligneola* and *Xylomeasma sordida* were generated in this study. Single spore isolates of *N. ligneola* and *X. sordida* were grown up in malt yeast broth (YM: 2% malt extract, 0.5% yeast extract, Biolab, Midrand, South Africa). Mycelium was collected in 2 mL Eppendorf tubes by centrifugation and freeze-dried. The lyophilized mycelium was ground to a fine powder and genomic DNA was extracted using the method described by Duong *et al.* (2013). A paired-end library was prepared (550 bp average insert sizes) for each species and sequenced using the Illumina HiSeq2500 (Macrogen Inc, Seoul, Korea). Quality and adapter trimming of the Illumina data was carried out using TRIMMOMATIC v0.36 (Bolger et al. 2014). *De-novo* genome assembly was done using SPADES v3.9 (Bankevich et al. 2012) and SSPACE-STANDARD v3.0 (Boetzer et al. 2011) was used for scaffolding. Assembly gaps were filled using GAPFILLER v1.10 (Boetzer & Pirovano 2012). The BENCHMARKING UNIVERSAL SINGLE-COPY ORTHOLOGS (BUSCO v2.0.1) tool was used to assess the completeness of each assembly against the Ascomycota dataset (Simão et al. 2015).

Results and Discussion

Supermatrix vs supertree analyses

There are two main approaches of phylogenetic reconstruction at the forefront of phylogenomic analyses: supermatrix analyses and supertree analyses. In both these approaches, primary character data forms the basis for the method, but they utilize this data in completely different ways to perform phylogenetic reconstruction (Bininda-Emonds 2004). In the supermatrix method, multiple sets of primary character data (e.g. sequence data) is combined into one large dataset – also known as the supermatrix. This supermatrix is then used as the source for phylogenetic analyses utilizing the researchers' tree reconstruction method of choice (De Queiroz & Gatesy 2007). In the supertree method each set of primary character data is analysed separately to produce multiple, small source trees. These source trees are then used as an input to reconstruct a larger, more robust phylogeny which summarizes

all the initial topologies (Bininda-Emonds 2005). Although in the past arguments were made for and against each method, in the genomics era both has its own benefits and drawbacks (Gatesy et al. 2002, Bininda-Emonds 2004, De Queiroz & Gatesy 2007). Therefore, instead of looking at these methods of phylogeny reconstruction as independent, they should rather be considered as complementary (Bininda-Emonds 2005, 2010).

In this work we utilized two different methods of phylogenetic tree reconstruction to determine the backbone for the Sordariomycetes: one supermatrix approaches (RaxML) and one supertree approach (STAG). The two phylogenies produced by the different analyses were highly congruent (**Supplementary Figures 1 & 2**), except for the Glomerellales clade. Supermatrix analyses using RaxML grouped this clade as a distinct sister lineage to both the Microascales and Hypocreales. However, in supertree analysis using STAG this clade formed a sister lineage to the Hypocreales. Although the grouping has high support in the supermatrix analyses, we still preferred the lower supported grouping of the supertree analyses. This was due to the fact that the supertree analyses included all orthologous genes shared for the species, whereas the RaxML analyses was based on 315 shared single-copy orthologous genes. Therefore, we consider the STAG analyses more robust as the data used to perform the analysis was more complete.

Nutrient acquisition by bark and ambrosia beetle associates

It has been known for quite some time that Ophiostomatoid fungi are unable to digest the woody constituents of their host (Valiev et al. 2009, Six 2019, Skelton et al. 2019). However, this leaves the question, if they don't get their nutrients from breaking down the woody tissue of the host, where do their nutrients come from? Studies investigating the histopathology and xylem dysfunction caused by blue-stain fungi have the answer to this question.

Studies investigating the progression of blue-stain fungi in pine (Ballard et al. 1982, 1984), spruce (Kristis & Offenthaler 2002, Kuroda 2005), oak (Kuroda, 2001, Takahashi et al. 2010), and others (Inch & Ploetz 2012, Kajii 2013) have all yielded similar results. What was shown, was that the fungi colonize the sapwood of the host by following the path of least resistance, initially entering the xylem vessels

through mechanical damage caused by the boring activities of their vectors (Ballard et al. 1984). From there, thick hyphae colonize the vessels vertically whilst fine hyphae spread transversely often entering the ray parenchyma cells by means of pit cells on the cell wall (Kuroda, 2001, Takahashi et al. 2010). Due to the large availability of nutrients found in both these cell types, the fungi can proliferate extensively within them (Ballard et al. 1984, Kajii 2013). Eventually this extensive colonization drains the cell of its contents, leaving only the necrotic, collapsed cell walls behind (Ballard et al. 1984).

Fungal colonization of the xylem vessels induced xylem dysfunction in the host. However, this process is not so much a result of the fungus but rather a symptom of the host response reaction. Fungal growth within the xylem and ray parenchyma induces secondary metabolite production by the host. These compounds (mainly tyloses) are sequestered by the host into these cells ahead of the infection in what is known as a reaction zone (Shain 1971, Kuroda 2005, Takahashi et al. 2010, Kajii 2013). Within this zone, the secondary metabolites accumulate in droplets within the cells. However, this accumulation leads to reduced sap-flow within the reaction zone, leaving the area dried out (Kuroda 2005). Formation of the reaction zone is usually enough the sequester further fungal colonization as growth of the fungus into the reaction zone does not typically occur unless the boring activities of the beetles carry them beyond it (Takahashi et al. 2010, Kajii 2013).

Comparative genomics annotation pipelines

Phylogenomic analyses

RaxML

#Get the shared single-copy BUSCO files
#Run BUSCO for all genomes using Ascomycota database. Allow BUSCO to select species for augustus. I did this using anaconda on a unix virtual box environment.
>for f in *.fa; do fn=${f/.fa/} run_BUSCO.py -i $f -l sordariomyceta_odb9 -o $fn -m genome -c 4; done
#Get all the shared single copy BUSCO's. For this you need faOneRecord installed.
#In folder with all runs:
>for f in $(ls|sed -e 's/run_//'); do sed -i 's/.fa.*//;s/:/ /' run_${f}/single_copy_busco_sequences/*;done
>for f in $(ls|sed -e 's/run_//'); do cat run_${f}/single_copy_busco_sequences/*.faa >> all.buscos; done
>mkdir full_table
>mv all.buscos full_table/
>for f in $(ls|sed -e 's/run_//'); do cp run_${f}/full_table* ./full_table; done
>cd full_table
>cat full_table* | grep -v "#" | grep "Complete" | cut -f1 | sort | uniq -c | awk '{if ($1 == "110 ") print $0;}' | awk -F " " '{print $2}' > single.copy.busco
>cat single.copy.busco | while read -r line; do faOneRecord all.buscos ${line} > ${line}.faa ; done
>mkdir ../busco.alignments
>mv *.faa ../busco.alignments

#Align single copy busco seqeunce datasets using PRANK
>ls *.faa | split -20 #split into smaller groups to run prank con-currently
#update split files to make them executable and to contain
>for f in x*; do sed -i 's/^/for f in /' ${f}; done
>for f in x*; do sed -i 's/faa/faa ; do fn=${f\/.faa\/} \&\& prank -d=${f} -o=${fn}\.faa; done/' ${f}; done
>for f in x*; do chmod u+x ${f}; done
#then execute these files concurrently

#run gblocks on alignments
for f in *.best.fas; do Gblocks ${f} -t=p -b=h; done
#fix the headers in the gblocks output files for future analyses
>for f in *-gb, do sed -i ‘s/ //g’ ${f}; done
#do PTP test using PUAP
#first convert the gblocks files to nexus format
>for f in *-gb; do fn=${f/.best.fas-gb} && gblocks2nexus.sh -g full -s protein ${f} ${fn}.nex; done
#even if protein is specified, gblocks2nexus.sh converts the file specification to DNA, so this needs to be fixed
>sed –i ‘s/=dna/=protein’ *.nex
#now write the paup block using vim and call the files PAUP_block.txt
begin PAUP;
Permute test = PTP nreps=10000 seach=heuristic;
end;

quit;
#add the PAUP_block.txt to each for the nexus files
>for f in *.nex; do cat ${f} PAUP_block.txt > ${f}_PAUP.nex; done

```
#run PTP test
>for f in *PAUP.nex; do PAUP4a163_ubuntu ${f} -l ${f}.log; done
#after PAUP has finished compare the P values of the different alignments and discard any that do not match the requirement
#Concatenate Gblocks files (-gb)


#run FASconCAT
>perl FASconCAT_v1.04.pl -n -p -l -s

#run partition finder using the FcC_supermatrix
>mkdir PartitionFinder
>cp FcC_supermatrix.phy PartitionFinder
#write the .cfg file
#get data matrix
>cat FcC_info.xls | grep EOG* | awk '{print $1 "\t" $2 "\t" $3}' | sort -n -k2.2 | awk '{print $1 "\t" = "\t" $2 ":" $3 ";"}' | sed -e 's/\./_/' > data_matrix
#to the top of the data_matrix file add
alignment= FcC_supermatrix.phy;
branchlengths= linked;
models= all;
model_selection= aicc;
[data_blocks]
#to the end of the data_matrix file add
[schemes]
search= rcluster;
#save the files as partition_finder.cfg and correct the : in the final file
>sed -i 's/:/-/'  partition_finder.cfg
#run partition finder
>PATH/TO/PROGRAM/partitionFinderProtein.py ./PartitionFinder --raxml --rcluster-max 1000 --rcluster-percent 10 -p 60
#Now RaxML using best partition scheme
raxmlHPC-PTHREADS-AVX2 -T 40 -f a -s FcC_supermatrix.phy -x 1234 -# 100 -m PROTGAMMAJTT -q best_scheme.txt -p 2 -o PFIJ,ABRA,SNOD -n Sordario_best_tree.tre
```

CAZyme extraction pipeline

```
#Run dbCAN2 online and download the prediction table for each annotation
#Extract the CAZymes that have at least two lines of evidence for each annotation
>for f in .*; do sed -e '/s/ ID/ID/' ${f} | awk '{$6>2 print}' | sed -e 's/([^)]*//g' -e 's/)//g' > ${f/.txt/}_filtered.txt; done
#manually work through the files and check for any mistakes. Save in .csv format
#get gene family only file
>for f in *.csv; do cut -d "," -f ${f} | sed -e 's/^CBM.*+//' | sed -e 's/+.*//;s/_.*//' > ${f/.filtered.txt/}.parsed ; done
#count the number of unique CAZyme families for each species
>for f in *.parsed; do sort ${f} | uniq -c | tr -s " " | awk '{print $2 "\t" $1}' > ${f/.parsed/}_cazyme.count.txt ; done
#get shared unique cazymes
```

>cat *_cazyme.count.txt | cat -f1 | sort | uniq > cazyme.list
#make a table file (cazyme.count.all)
#copy information for each species into the table. [sp] – insert the name for the species file
Map_data_ids [sp]_cazyme.count.txt cazyme-count-all - col #

Lipase identification pipeline

#Download the LED database content file: led.tar.gz. Combine hmm files within this folder to make hmmsearch database
> cat *.hmm > LED.hmm
#Run HMMsearch - I did this in anaconda environment with hmmer installed
#Use protein evidence files (.faa)
#Set e-value (-E) to 1E-4 (0.0001). Make a directory (outputs) for results.
> for f in *.faa; do fn=${f%.faa} && hmmsearch --cpu 8 -E 0.0001 --tblout outputs/${fn}.targets.tab --domtblout outputs/${fn}.domains.tab LED.hmm ${f} >> outputs/${fn}.hmm.out; done
#This takes a while

#Extract best hits from *.target.tab files.
#Remove headers & footers from hmmer and sort that best hits of each protein appears first and print to new file. Make directory best.hits for output.
>for f in *.target.tab; do fn=${f%.target.tab} && grep -v "#" ${f} | sort -k1,1 k6,6gr -k5,5g | sort -u -k1,1 > ./best.hits/${fn}.besthits.txt; done
#Extract lipase families (need lipase.families.list file – list containing all lipase family names one beneath the other. Available supplementary file.)
#Make directory for outputs (strict.lipases)
> for f in *.txt; do fn=${f%.besthits.txt} && grep -F -f lipase.families.list ${f} > ./strict.lipases/${fn}.strict.lipases; done

#Extract cutinases
#Make directory for output (strict.cutinases)
> for f in *.txt; do fn=${f%.besthits.txt} && grep "abH36" ${f} > ./strict.cutinases/${fn}.strict.cutinases

#Get individual family counts (do for each individual family)
>grep -c "abH01" *.strict.cutinases >> abH01.count
#Copy values into excel table

Peptidase identification pipeline

#Download Merops_scan.lib from Merops database
#Convert to Merops_scan.lib fasta format
Blast maker annotated protein files against Merops.scan.fa

```
>makeblastdb -in merops_scan.lib -dbtype prot
>for f in *.faa; do blastp -db merops_scan.lib -query ${f} -out ${f}.blast.out -evalue 0.0001 -outfmt 6 -num_threads 4; done
```

```
#Sort BLAST results
>for f in *.out; do fn=${f%.faa} && sort -k1,1 -k12,12gr -k11,11g -k3,3gr ${f} | sort -u -k1,1 --merge >> ${f}.merops.best; done
#To get total counts just count the lines
>wc -l
```

#To get individual sub-family counts -> firstly make a table with all the merops accessions in one column and their respective sub-family in the one next to it. (Available supplementary file)

```
#make a file called merops.awk containing
BEGIN{OFS = "\t"}
        {if (NR == FNR){
        Merops [$1] = $0
        }
        else{
                if($2 in merops){
                print $1, $2, merops [$2]
                }
        }
}
#Then get all the accessions from your analyses
>for f in *.best; do fn={$f%.merops.best} && awk '{print $1, $2}' ${f} >> ${fn}.accession; done
#Then import the sub-family names into your analyses
>for f in *.accession; do fn=${f%.accession} && awk merops.awk merops_family.tab ${f} >> ${fn}.merops.families ; done#inside the merops.awk file:
#Print a file with the different sub-families contained in each individual analyses
>for f in *.families; do fn${f%.merops.families} && awk '{print $4}' ${f} >> ${fn}.families.list; done
#If you now want to know only the families - print just the first letter of each family name which specifies to which family each protein belongs
>for f in *.list; do fn=${f%.families.list} && cut -c -1 ${f} >> ${fn}.1char ; done
#you can now count the number of times each family appears using grep. E.g. for glutamic G" family
>grep -c "G" *.1char > ./Glutamic.count
#For subfamily counts : I did this in excel.
```

Secondary metabolite gene cluster identification

```
#Antismash is available through bioconda to be installed in an anaconda environment
#Installation instructions can be found online at https://docs.antismash.secondarymetabolites.org/install/
#a short antismahs run was done as (using the genome files):
>for f in *.fas; do antismash -c 6 ${f}; done
```

#Outputs were manually inspected and numbers copied to an excel file

Secreted protein identification

#Due to a problem with the annotation software, I first had to remove the lines from my fasta files. I also added sequential numbering for the proteins to make them easier to identify in the next step.

#From this
>AACR_1053314
MVVSKTRLCPLFTRRHIEGMIAEGHSITIVNQYVLKMDGWLKFHPGGDKAIMHMVGRDATDEVTALHSPKAMDQMIRYRIGRIEG
RWKNFIPPIQGGHFRLPTAVDGTDANQDERAHSTKPDAAAWCEPASFSSSVPSSRDTSPVFDVDKSFRRRRGTAPGSCPTSTTSVST
EEDGMHELTPMGFLDAETRKAINLDLDQYPPLDSETQANIIRKYRQLHERIKAEGLYDCNYSSYAIEVSRYCLLMFGVLFFLRCGW
YVTSAACLGALWHQLVFTAHDAGHMGITHDFHIDTCIGIFAANFIGGLSIGWWKRSHNVHHIVTNSPEHDPDIEHMPFLAVSHRFF
GDLYSTYYERLMKYDAFAKVLLSVQSRMFYIIMLFGRFNLYVLSWDHLIFRRGPRKGPAWWHWWLEVAGQVFFWTWFGYGVL
YRCIPTAWYRVIYVLVSHMLQAPVHIQITLSHFAMSTADLGPDESFPQRMLRTTMDIDCPTWLDFFHGGLQFQAIHHLYPRLPRHN
LRRTQKLVQEFCNDVGIPYALYGFVDSNKEVIGRLAEVSRQAAILAECQRVVAGDT
>AACR_2023495
MCFALRKPLLCRKMGAKTQRGAHAKPPENSEVARAPAAQSEPGPSKQPAQTTQPHTEPPTKPRAQPQQRTQTTKSTQRPIPTQQN
RPGRPSRRGSPEPPNSTPDPAPPSPTFPKTARDGEGDITMEDADSDDDSDEIARYKTPTQASKRDRRKPPWIPL

#add sequential numbering to the fasta header so all are the same length && #replace the un-numbered line with numbers
>for f in *.faa; do fn=${f%.faa} && awk '/_.*/{n=sprintf("%04d",++i) sub(/_.*/,"|" n "|")}1' ${f} >> ${fn}.num; done
>for f in *.num; do sed -i 's/||/|00000|/' ${f}; done

#to this
>AACR|00011|
MVVSKTRLCPLFTRRHIEGMIAEGHSITIVNQYVLKMDGWLKFHPGGDKAIMHMVGRDATDEVTALHSPKAMDQMIRYRIGRIEG
RWKNFIPPIQGGHFRLPTAVDGTDANQDERAHSTKPDAAAWCEPASFSSSVPSSRDTSPVFDVDKSFRRRRGTAPGSCPTSTTSVST
EEDGMHELTPMGFLDAETRKAINLDLDQYPPLDSETQANIIRKYRQLHERIKAEGLYDCNYSSYAIEVSRYCLLMFGVLFFLRCGW
YVTSAACLGALWHQLVFTAHDAGHMGITHDFHIDTCIGIFAANFIGGLSIGWWKRSHNVHHIVTNSPEHDPDIEHMPFLAVSHRFF
GDLYSTYYERLMKYDAFAKVLLSVQSRMFYIIMLFGRFNLYVLSWDHLIFRRGPRKGPAWWHWWLEVAGQVFFWTWFGYGVL
YRCIPTAWYRVIYVLVSHMLQAPVHIQITLSHFAMSTADLGPDESFPQRMLRTTMDIDCPTWLDFFHGGLQFQAIHHLYPRLPRHN
LRRTQKLVQEFCNDVGIPYALYGFVDSNKEVIGRLAEVSRQAAILAECQRVVAGDT
>AACR|00021|
MCFALRKPLLCRKMGAKTQRGAHAKPPENSEVARAPAAQSEPGPSKQPAQTTQPHTEPPTKPRAQPQQRTQTTKSTQRPIPTQQN
RPGRPSRRGSPEPPNSTPDPAPPSPTFPKTARDGEGDITMEDADSDDDSDEIARYKTPTQASKRDRRKPPWIPL

#remove new lines from file
for f in *num; do fn=${f%.num} && tr '\n' ' ' < $f > $fn.nolines; done
#insert newline between sequences
for f in *nolines; do sed -i 's/>/\n>/g' ${f}; done
#remove spaces
for f in *nolines; do sed -i 's/ //g' $f; done
#add space between second pipe and sequence
for f in *nolines; do sed -i 's/|/| /2' ${f}; done

#you get this
>AACR|00011|
MVVSKTRLCPLFTRRHIEGMIAEGHSITIVNQYVLKMDGWLKFHPGGDKAIMHMVGRDATDEVTALHSPKAMDQMIRYRIGRIER
WKNFIPPIQGGHFRLPTAVDGTDANQDERAHSTKPDAAAWCEPASFSSSVPSSRDTSPVFDVDKSFRRRRGTAPGSCPTSTTSVSTE
DGMHELTPMGFLDAETRKAINLDLDQYPPLDSETQANIIRKYRQLHERIKAEGLYDCNYSSYAIEVSRYCLLMFGVLFFLRCGWYT
SAACLGALWHQLVFTAHDAGHMGITHDFHIDTCIGIFAANFIGGLSIGWWKRSHNVHHIVTNSPEHDPDIEHMPFLAVSHRFFGDY
STYYERLMKYDAFAKVLLSVQSRMFYIIMLFGRFNLYVLSWDHLIFRRGPRKGPAWWHWWLEVAGQVFFWTWFGYGVLYRCIT

AWYRVIYVLVSHMLQAPVHIQITLSHFAMSTADLGPDESFPQRMLRTTMDIDCPTWLDFFHGGLQFQAIHHLYPRLPRHNLRRTKL
VQEFCNDVGIPYALYGFVDSNKEVIGRLAEVSRQAAILAECQRVVAGDT
>AACR|00021|
MCFALRKPLLCRKMGAKTQRGAHAKPPENSEVARAPAAQSEPGPSKQPAQTTQPHTEPPTKPRAQPQQRTQTTKSTQRPIPTQQRP
GRPSRRGSPEPPNSTPDPAPPSPTFPKTARDGEGDITMEDADSDDDSDEIARYKTPTQASKRDRRKPPWIPL

#pull out seqeunces of 200 AA or shorter (this can now be done because all headers are of equal length of 13 char)
for f in *nolines; do fn=${f%.nolines} && grep -x '.\{0,213\}' ${f} >> $fn.signalP ; done

#All the following annotations can be done either using the online server or the downloaded versions of the annotation tools. The different programs can be downloaded for academic use at **SignalP** (http://www.cbs.dtu.dk/cgi-bin/nph-sw_request?signalp), **TargetP** (http://www.cbs.dtu.dk/cgi-bin/nph-sw_request?targetp) & **TMHMM** (www.cbs.dtu.dk/cgi-bin/nph-sw_request?tmhmm). Follow the installation instructions and execute to generate short annotation files. Start with your protein annotation file (*.faa) → E.g. for signalP
>for f in *.faa; do ./signal -fasta ${f} -format short -batch 10000; done

#Once you have all the annotation files determine the proteins that have two or more lines of evidence
#Pull out the list of secreted proteins for each species from each annotation – I created an output folder for each annotation tool containing all the output files produced by the run
###signalP #
>for f in *.short.out*; do fn=${f%.short.out.signalP} && grep “SP” ${f} | awk ‘{print $1}’ | sed -e ‘s/_/|/’ >> ${fn}.signalP.list; done
###targetP#
#Firstly pull out the secreted proteins – there is a double space in front and behind the S.
>for f in *; do fn=${f%.targetP.out} && grep “ S “ ${f} >> ${fn}.targetP.secreted; done
Second get a list of the secreted proteins – there is a triple space after the protein name
>for f in *.secreted; do fn=${f%.targetP.secreted} && sed -e ‘s/ .*//’ ${f} >> ${fn}.targetP.list; done
###TMHMM#
>for f in *.TMHMM.out; do fn=${f%.TMHMM.out} && grep “ExpAA=0.00” ${f} && awk ‘{print $1}’ >> ${fn}.TMHMM.nodomains; done
#Count the number of secreted proteins – below the ${f}.* refers to the signalP, targetP, and TMHMM file for each species. (add a space after the one and avoid pulling out protein names).
>cat ${f}.* | sort | uniq -c| grep -v “1 “ > ${f}.secreted.proteins
>wc -l *.secreted.proteins

#now to get the small secreted proteins (smaller than 200AA)
#first make a directory containing the files with list of secreted proteins
>mkdir small.sec.prot
>mv *.secreted.proteins small.sec.proteins
#now for each species make their own folder and move the secreted protein files into them
>for f in *.secreted.proteins; do fn=${f%.secreted.proteins} && mkdir ${fn} && mv ${f} ${fn}; done
#for the files containing the small proteins we made earlier - pull out the names of the proteins
>for f in *.small.proteins; do fn=${f%.small.proteins} && grep ‘>’ ${f} >> ${fn}.SP.names; done

#fix file names to match those in secreted protein list
>for f in *names; do sed -i 's/>//' && do sed -i 's/|//2' ${f}; done
#move the files from their current location to their correct species directories
>for f in *small.proteins; do fn=${f%.small.proteins} && do mv ${f} PATH/TO/DIR/small.sec.prot/${fn}; done
#now count the number of proteins shared between the two files in these directories
>for f in *; do cd ~/PATH/TO/DIR/small.sec.prot/${f} && cat ${f}* | sort | uniq -c | grep "2 "| wc -l >> ${f}.count && cd ~/PATH/TO/DIR/small.sec.prot; done
#move the count files to their own directory
>mkdir SSP.count
> for f in *; do cd ~/PATH/TO/DIR/small.sec.prot/${f} && mv ${f}.count ../SSP.count && cd ~/PATH/TO/DIR/small.sec.prot; done
#Combine these files keeping the file header
>grep "" *.count >> SSP.totals

References

Ballard, R.G., Walsh, M.A., & Cole, W.E. (1982). Blue-stain fungi in xylem of lodgepole pine: A light-microscope study on extent of hyphal distribution. *Canadian Journal of Botany*, 60, 2334-2341. DOI: https://doi.org/10.1139/b82-285

Ballard, R.G., Walsh, M.A., & Cole, W.E. (1984). The penetration and growth of blue-stain fungi in the sapwood of lodgepole pine attacked by mountain pine beetle. *Canadian Journal of Botany*, 62, 1724-1729. DOI: https://doi.org/10.1139/b84-233

Bankevich A., Nurk S., Antipov D., Gurevich A.A., & Dvorkin, M. (2012). SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology*, 19, 455-477. DOI: https://doi.org/10.1089/cmb.2012.0021

Bininda-Emonds, O.R. (2004). Trees versus characters and the supertree/supermatrix "paradox". *Systematic Biology*, 53, 356-359.

Bininda-Emonds, O.R. (2005). Supertree construction in the genomic age. *Methods in Enzymology*, 395, 745-757. DOI: https://doi.org/10.1016/S0076-6879(05)95038-6

Bininda-Emonds, O.R. (2010). The future of supertrees: Bridging the gap with supermatrices. *Palaeodiversity*, 3, 99-106.

Boetzer, M., Henkel, C.V., Jansen, H.J., Butler, D., & Pirovano, W. (2011). Scaffolding pre-assembled contigs using SSPACE. *Bioinformatics*, 27, 578-579. DOI: https://doi.org/10.1093/bioinformatics/btq683

Boetzer, M, & Pirovano, W. (2012). Toward almost closed genomes with GapFiller. *Genome Biology*, 13, R56. DOI: https://doi.org/10.1186/gb-2012-13-6-r56

Bolger, A.M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, 30, 2114-2120. DOI: https://doi.org/10.1093/bioinformatics/btu170

De Queiroz, A., & Gatesy, J. (2007). The supermatrix approach to systematics. *Trends in Ecology & Evolution*, 22, 34-41. DOI: https://doi.org/10.1016/j.tree.2006.10.002

Duong, T.A., De Beer, Z.W., Wingfield, B.D., & Wingfield, M.J. (2012). Phylogeny and taxonomy of species in the *Grosmannia serpens* complex. *Mycologia*, 104, 715-732. DOI:https://doi.org/10.3852/11-109

Gatesy, J., Baker, R.H., & Hayashi, C. (2004). Inconsistencies in arguments for the supertree approach: Supermatrices versus supertrees of *Crocodylia*. *Systematic Biology*, 53, 342.

Inch, S.A., & Ploetz, R.C. (2012). Impact of laurel wilt, caused by *Raffaelea lauricola*, on xylem function in avocado, *Persea americana*. *Forest Pathology*, 42, 239-245. DOI: https://doi.org/10.1111/j.1439-0329.2011.00749.x

Kajii, C., Morita, T., Jikumaru, S., Kajimura, H., Yamaoka, Y., & Kuroda, K. (2013). Xylem dysfunction in *Ficus carica* infected with wilt fungus *Ceratocystis ficicola* and the role of the vector beetle *Euwallacea interjectus*. *IAWA Journal*, 34, 301-312. DOI: https://doi.org/10.1163/22941932-00000025

Kirisits, T., & Offenthaler, I. (2002). Xylem sap flow of Norway spruce after inoculation with the blue-stain fungus *Ceratocystis polonica*. *Plant Pathology*, 51, 359-364. DOI: https://doi.org/10.1046/j.1365-3059.2002.00722.x

Kuroda, K. (2001). Responses of *Quercus* sapwood to infection with the pathogenic fungus of a new wilt disease vectored by the ambrosia beetle *Platypus quercivorus*. *Journal of Wood Science*, 47, 425-429. DOI: https://doi.org/10.1007/BF00767893

Kuroda, K. (2005). Xylem dysfunction in Yezo spruce (*Picea jezoensis*) after inoculation with the blue-stain fungus *Ceratocystis polonica*. *Forest Pathology*, 35, 346-358. DOI: https://doi.org/10.1111/j.1439-0329.2005.00424.x

Shain, L. (1971). The response of sapwood of Norway spruce to infection by *Fomes annosus*. *Phytopathology*, 61, 301-307.

Simão, F.A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E.V. & Zdobnov, E.M. (2015) BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*, 31, 3210-3212. DOI: https://doi.org/10.1093/bioinformatics/btv351

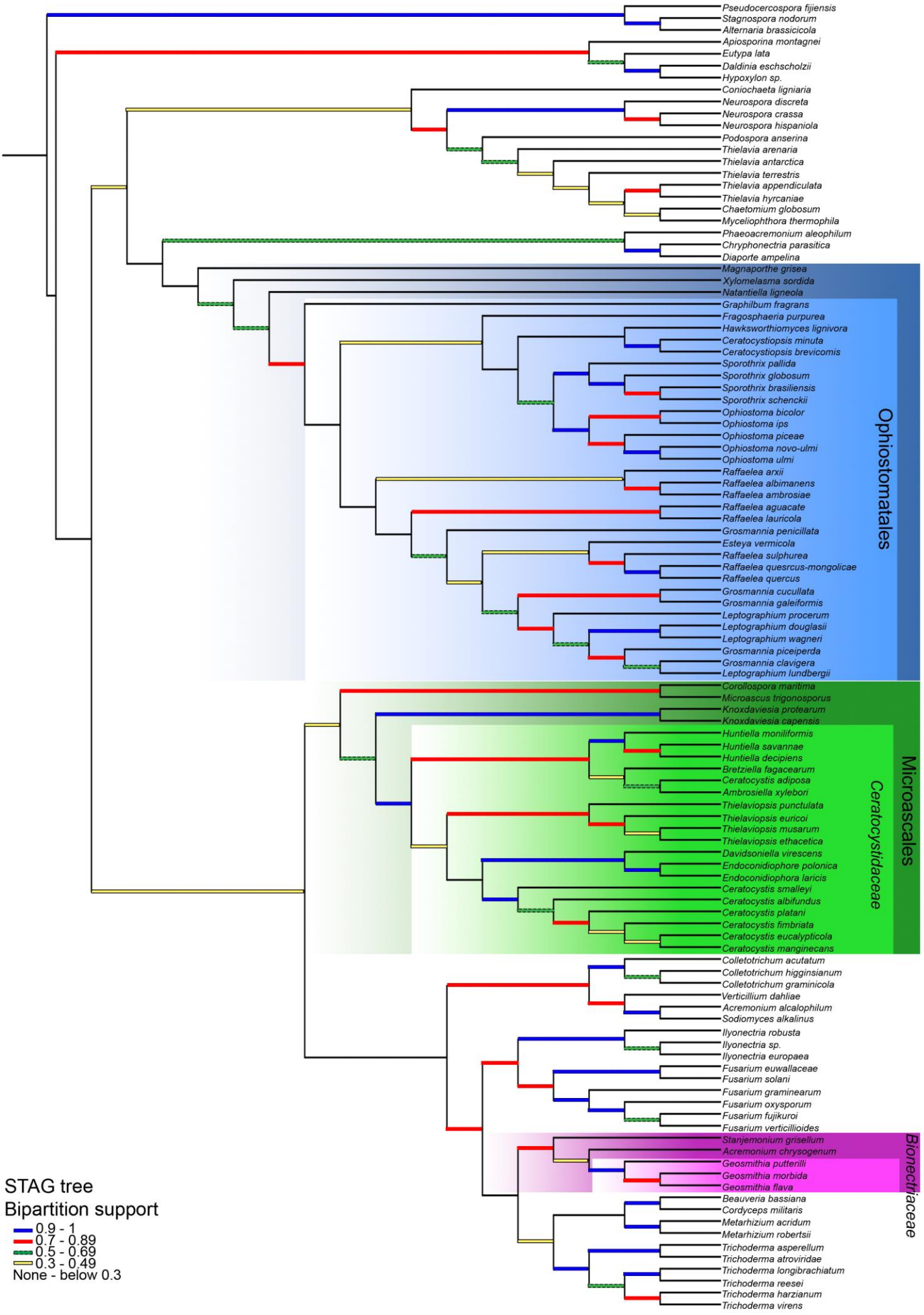
Six DL (2019) A major symbiont shift supports a major niche shift in a clade of tree-killing bark beetles. *Ecological Entomology*, 45, 190-201. DOI: https://doi.org/10.1111/een.12786

Skelton, J., Jusino, M.A., Carlson, P.M., Smith, K., Banik, M.T., et al. (2019) Relationships among wood-boring beetles, fungi, and the decomposition of forest biomass. *Molecular Ecology*, 28, 4971-4986. DOI: https://doi.org/10.1111/mec.15263

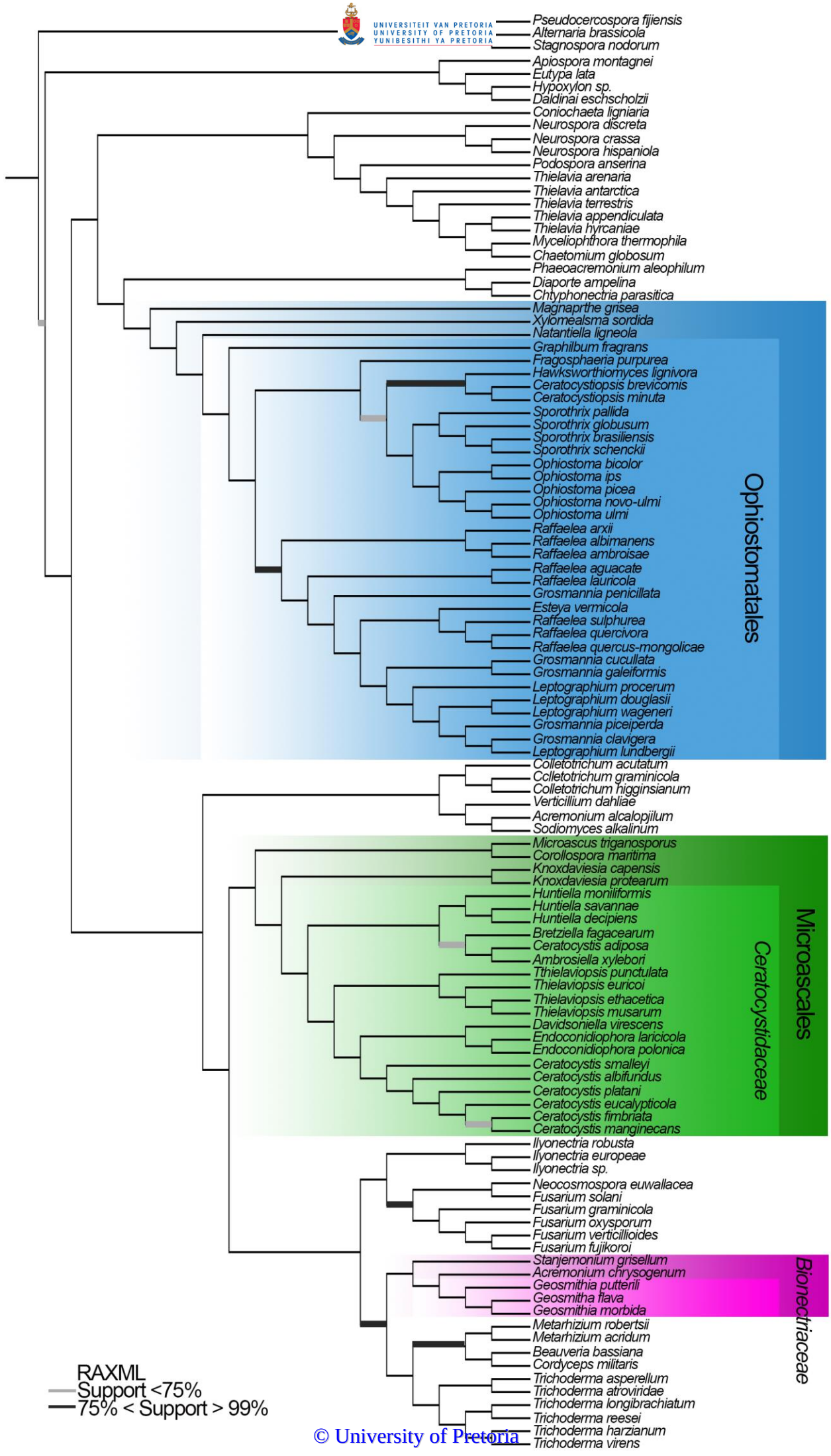
Takahashi, Y., Matsushita, N., & Hogetsu, T. (2010). Spatial distribution of *Raffaelea quercivora* in xylem of naturally infested and inoculated oak trees. *Phytopathology*, 100, 747-755. DOI: https://doi.org/10.1094/PHYTO-100-8-0747

Valiev, A., Ogel, Z.B. & Klepzig, K.D. (2009) Analysis of cellulase and polyphenol oxidase production by southern pine beetle associated fungi. *Symbiosis*, 49, 37-42. DOI: https://doi.org/10.1007/s13199-009-0007-0

Supplementary Figure 1. Phylogram produced by supertree analysis of all orthologous genes using STAG. Nodes with bipartition support between 0.9 and 1 are indicated in blue; with support between 0.7 and 0.89 in red; with support between 0.5 and 0.69 in green; with support between 0.3 and 0.49 in yellow; and below 0.3 are not indicated.



Supplementary Figure 2. Phylogram produced by supermatrix analyses of 315 shared, single-copy orthologous BUSCO genes using RaxML. Branches with support lower than 75% are indicated with bold grey branched; with support between 75% and 99% are indicated with bold black branches; all other branches had full support.



SUPPLEMENTARY CHAPTER

A checklist of South African bark and ambrosia beetles

(Coleoptera: Curculionidae: Scolytinae, Platypodinae)

A checklist of South African bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae, Platypodinae)

1. Abstract

Bark and ambrosia beetles are small wood-boring insects (Coleoptera: Curculionidae) that have evolved sophisticated symbioses with fungi. Since the start of the 21st century, the increasing accidental introductions of these insects and their symbiotic fungi has become a major problem for forest and timber industries, especially in the Northern Hemisphere. Consequently, they have been extensively studied in this region and their species diversities are relatively well known. In contrast, these pests have attracted relatively little research interest in the Southern Hemisphere. In this study we sought to address this knowledge gap by creating and updated checklist of bark and ambrosia beetle diversity in South Africa. More than 200 species of bark and ambrosia beetles are shown to be present in the country, 24 of which represent first reports.

2. Introduction

In an increasingly interconnected society, the process of globalization is facilitating the spread of invasive pests and pathogens more than ever before (Meyerson & Mooney 2007, Pyšek et al. 2020, Jactel et al. 2020, Wingfield et al. 2020). As a result, the movement of unregulated or improperly treated plant, wood and other biological material poses a substantial threat to sustainable agriculture and forestry world-wide (Wingfield et al. 2008, 2015, Hurley et al. 2016, 2017, Paini et al. 2016, Grousset et al. 2020, Paap et al. 2020). One of the most frequently encountered insect groups moved in this way are bark and ambrosia beetles (Haack 2001, 2006, Brockerhoff et al. 2006, Meurisse et al. 2019, Faccoli et al. 2020). The ease of movement of these insects is most often attributed to their small size, making them inconspicuous and easily disseminated in lumber, wood products and packaging (Aukema et al. 2011, Liebhold et al. 2012).

Bark and ambrosia beetles represent a polyphyletic assemblage of Coleopteran species that reside in two sub-families of the Curculionidae i.e. Scolytinae and Platypodinae (Hulcr et al. 2015). These natural

forest inhabitants are often considered harmless because they primarily infest stressed, dying or recently dead trees in their native ranges (Hulcr & Stelinski 2017, Hulcr et al. 2017). However, dissemination of these insects together with their symbiotic fungal partners into non-native environments has resulted in some of the most devastating forest and tree related disease outbreaks ever recorded, such as the cases of Dutch elm disease and Laurel wilt (Ploetz et al. 2013, Hulcr & Stelinski, 2017). Although most introductions do not result in such devastation, boring activities of the beetles reduce timber value and sap-stain of lumber induced by the growth of their associated fungi can result in substantial economic losses to global forest industries annually (Seifert 1993, Gregoire et al. 2015, Boland et al. 2016, Susaeta et al. 2016).

In many Southern Hemisphere countries, studies focussed on bark and ambrosia beetles are relatively uncommon (Landi et al. 2017, Gomez et al. 2020). A small number of studies are available from South America (Wood 2007), Australia (Dole & Beaver 2008, Mitchell & Maddox 2010, Bickerstaff et al. 2020), and New Zealand (Brokerhoff et al. 2003, 2006), but these mostly focussed on introduced and problematic species. Even fewer studies have been published from Southern African countries, and most of these are reports from South Africa. Very few investigations focused on the diversity of bark and ambrosia beetles have been conducted in South Africa subsequent to the work of the Austrian naturalist Karl Schedl who published extensive species lists of collected specimens (Schedl 1955, 1957, 1960, 1965, 1970, 1975, 1982). Additionally, only a single complete record of species found in the country exists and forms part of the global catalogue produced by Wood & Bright (1992). Since that time, only a few new records of these insects have been made (Six et al. 2005, Jordal 2009, Paap et al. 2018, Van Der Linde et al. 2016, 2018, Nel et al. 2020).

In general, insect pests affecting natural and plantation forestry in South Africa are treated very differently from one another. Plantation forest often constitute large ranges of non-native trees, with relatively low insect pests in areas of cultivation. Therefore, the accidental appearance of a new pest or disease often results in serious damage and warrants investigation. In contrast, natural forests rarely attract much research interest as damage resulting from pest introduction is much less conspicuous (Wingfield et al. 2020). Because of the difference in research interest in these areas, pest species

affecting South African plantation forestry has been quite well studied and reviewed (Bush et al. 2016, Roux et al. 2012, Hurley et al. 2017), whereas little is known about what is happening in our natural forest areas (Wingfield et al. 2020).

The first aim of this study was to provide an updated checklist of bark and ambrosia beetle species present in South Africa. Lists of species records were compiled from the published literature (Schedl 1957, 1960,1 965, 1970, 1975, 1982, Wood & Bright 1992, Mandelshtam & Beaver 2003, Six et al. 2005, Alonzo-Zarazaga & Lyal 2009, Jordal 2009, Bright 2014, 2021, Van Der Linde et al. 2016, 2018, Paap et al. 2018, Nel et al. 2020) and from inspections of Scolytinae and Platypodinae species preserved in entomological collections. In addition, specimens collected from field surveys conducted during the summers from September 2018 - December 2019 and those submitted to the Diagnostic Clinic of the Forestry and Agricultural Biotechnology Institute (FABI) were included. An additional aim of this study was to generate sequence data for as many of the collected species as possible to build up currently available databases on these insects.

3. Materials & Methods

3.1. Entomological collections

During November 2019, Scolytinae and Platypodinae specimens housed in two entomological collections of South Africa were examined. These included the Coleopteran collection of the Ditsong (previously Transvaal) Museum of Natural History, Pretoria, South Africa (https://ditsong.org.za) and the South African National Collection of Insects (SANCI) of the Agricultural Research Council (ARC) Biosystematics Division, Pretoria, South Africa (http://www.arc.agric.za/arc-ppri/Pages/Biosystematics/National-Collection-of-Insects.aspx). Specimens deposited under valid species names (or their synonyms) were presented. All specimens collected from South Africa and housed in these collections were examined and included in this study. These collections were considered the most important to examine as they house all the type specimens of species described from South Africa. Additionally, the Ditsong museum houses most of the specimens collected by Schedl during his visits to South Africa. Many of the species housed at the Ditsong collection have also had their identities

confirmed by Prof. Roger A. Beaver (retired- Chiang Mai, Thailand) aiding in their credibility of their identifications (Ruth Muller, personal communication – Curator of the coleoptera collection, Ditsong Museum, Pretoria, South Africa (muller@ditsong.org.za)).

3.2. Specimen collections

South Africa includes many different biomes and ecoregions throughout the country. Limpopo is primarily dominated by montane grasslands and shrublands (Olson et al. 2001), but different forest ecosystems occur along the mountainous escarpments. Two locations, representing distinct ecoregions in Limpopo were selected to survey ambrosia beetles in this study. The first location (located 23° 42' 29.491" S, 30° 5' 57.638" E) was dominated by humid Afromontane forest and the second location (located 23° 44' 29.491" S, 30° 11' 15.417" E) was dominated by dry bushveld (grasslands) and shrubland. Field collections were carried out using the Bambara beetle trap (Hulcr & McCoy, 2015) during the periods of September 2018 – April 2019 and September 2019 – December 2019. Additionally, woody material infested with woodborers that were submitted to the Diagnostic Clinic based at FABI were placed in emergence chambers and the emerging beetles were collected. Specimens were maintained in 96% ethanol at -20°C in the bark and ambrosia beetle collection at FABI, University of Pretoria, South Africa overseen by Prof. Wilhelm de Beer until morphological examination and DNA sequencing could be conducted. After being used for DNA extraction, the remaining specimens were returned to the beetle collection.

3.3. Morphological observations

Whole specimens were examined and identified morphologically using a Zeiss Discovery V12 stereomicroscope (Zeiss, Oberkocken, Germany) with a Zeiss Axiocam IcC 5 camera. The ventral, lateral and dorsal aspects of specimens were examined up to 100X magnification. Keys of Rabgalia et al. (2006), Faccoli (2008), and Gomez et al. (2018) were used to aid in initial genus level identification. Identification involved comparisons of antennal club shape, elytral shape and morphology, and pronotum morphology. Focus-stacked photographs were produced for these specimens using HELICON FOCUS V5 (HeliconSoft, Kharkiv, Ukraine) with up to 50 different images. For species collected during

the surveys in this study, representative specimens were sent to Dr. Andrew Johnson (University of Florida) or Prof. Roger A. Beaver for confirmation of species identifications.

3.4. DNA extraction, PCR and sequencing

In some cases, the obtained specimens were damaged during collection and could not be used for morphological identification. DNA extraction and sequencing were performed to identify these specimens. Additionally, representative specimens of all species collected in this study were also selected and included for DNA sequencing to build up currently available sequence databases for these insects. For whole specimens, DNA extraction was performed from the heads of individual beetles that were aseptically removed using tweezers. Where collected specimens were broken, all available material was used for DNA extraction. Extractions were performed using the Macherey Nagel NucleoSpin Tissue kit (Macherey-Nagel, Dueren, Germany) following the manufacturer's protocol, except for the final elution volume which was reduced to 60 µl.

PCR amplification and sequencing of the mitochondrial cytochrome oxidase 1 (COI) was done using the universal primer pair LCO1490 & HCO2198 (Folmer et al., 1994). Additional amplification of the 28S ribosomal large subunit (28S) and carbomyl-phosphate synthetase 2 (CAD) regions were carried out using the primer pairs 3665 & 4068 (Belshaw & Quicke 1997, Cognato 2013) and apCADforB2 and apCADrevlmod (Cognato 2013), respectively. PCR reactions were carried out as described by Cognato (2013) in 25 µL reaction volumes. Two microliters of the extracted DNA was used as template for PCR amplification.

PCR products were treated with EXOSAP-IT™ PCR PRODUCT CLEANUP REAGENT (ThermoFisher Scientific, Massachusetts, United States) to remove any residual dNTPs and primers. Sequencing PCR reactions were carried out for both the forward and reverse strand using the same primers as used in PCR according to THE BIGDYE® TERMINATOR V3.1 Cycle sequencing protocol (ThermoFisher Scientific, Massachusetts, United States). All sequencing PCR reactions were performed using an annealing temperature of 55°C. Sequencing PCR products were purified using the sodium acetate and ethanol precipitation method (Maniatis et al. 1982) before submission to the DNA sanger sequencing

facility based at the University of Pretoria for fragment analyses using an ABI PRISM®3500 GENETIC ANALYZER (Applied Biosystems, California, United States). The software SEQUENCE SCANNER V1.0 (https://sequence-scanner-software.software.informer.com/) was used for sequence quality assessment and editing. Contigs were assembled using CONTIGEXPRESS v11 (Science Express, New York, United States). Newly obtained sequences will be deposited in NCBI GenBank. Generated consensus sequences were used in BLASTN searches against the non-redundant nucleotide sequence database available in NCBI GenBank to confirm putative morphological identifications of species.

4. Results

In total, from all records considered, 24 Platypodinae species (**Table 1**) and 236 Scolytinae species (**Table 2**) were identified from South Africa. These included two Platypodinae and 22 Scolytinae species reported from the country for the first time (**Table 1 & 2**). Physical specimens for four of the newly recorded species were collected during this study.

Field expeditions and beetle rearing from material submitted by members of the public yielded 2348 beetle specimens. Of these, 2139 specimens were identified to species level. These included three Platypodinae species and 37 Scolytinae species (**Tabke**. The remaining specimens were either too damaged to be successfully identified or might represent novel taxa (having morphologies that do not match those of any of the known species) and thus were not considered in the current study.

4.1. Field collected specimens

In total 1897 specimens were collected during field expeditions to Tzaneen, Limpopo (**Figure 1A**). These represent 683 specimens collected from the grassland ecoregion and 1214 specimens collected from the Afromontane ecoregion. Of the 1897 specimens collected, 1794 were identified to species level using morphological data. The remaining 103 specimens could not be identified to species level but were identified to either genus or family level. Results for the unidentified specimens are not included in this checklist and will be considered at a later date.

In total 26 species were collected from the two locations. Sixteen species were collected from the grassland ecoregion and 24 species were collected from the Afromontane ecoregion (**Figure 1B**). Of the 26 species collected, 14 were collected from both sites i.e. *Ambrosiodmus pithecolobius, Doliopygus erichsoni, Eccoptopterus spinosus, Glostatus squamosus, Hypothenemus eruditus, Hypothenemus hampei, Premnobius cavipennis, Xyleborinus saxesenii, Xyleborus affinis, Xyleborus ferrugineus, Xyleborus perdiligens* and *Xylosandrus crassiusculus. Hypothenemus crudiae, H. setosus* and *H. seriatus* were collected only from the grassland ecoregion, whereas *Amphiscolytus capensis, Ambrosiodmus tachygraphus, Eidophelus onyanganus, Euwallacea xanthopus, Hapalogenius fuscipennis, Scolytoplatypus fasciatus, Strombophorus capensis, Xyleborinus aemulus, Xyleborus antaisaka* and *Xyloctonus scolytoides* were collected only from the Afromontane ecoregion. The four most dominant species collected in this study were *Xyleborinus saxesenii* (30%), *Scolytoplatypus fasciatus* (23%), *Hypothenemus eruditus* (11%) and *Premnobius cavipennis* (10%). Despite being the second most abundant species collected, *Scolytoplatypus fasciatus* was found only in the Afromontane ecoregion and represented 36% of the specimens collected from this location.

4.2. Submitted specimens

Various samples of infested wood and collected insect specimens were submitted to the FABI Diagnostic clinic for beetle identification. Submissions were made by both private homeowners and foresters. From these samples, 242 specimens of bark and ambrosia beetle were collected. From these specimens, 22 different species were identified (**Table 3**).

4.3. Entomological collections

A total of 163 species housed at two entomological collections visited were considered in this study, of these 109 were at the Ditsong Museum and 103 were at SANCI. These collections house specimens for 21 species that had been identified and submitted but had not been reported (**Table 1 & 2**). From the 163 species identified, 41 species have specimens available at both collections.

4.4. DNA sequencing

Of the 40 species for which specimens were collected in this study, we were able to obtain DNA sequence data for one or more regions for 38 species (**Table 4**). For 33 species we were able to generate sequence data for two or more of the selected regions. For six of these species i.e. *Amphiscolytus capensis, Hypothenemus crudiae, Hapalogenius fuscipennis, Ambrosiodmus pithecolobius, Xyleborus antaisaka* and *Xyleborus perdiligens*, this was the first time that any DNA sequence data has been generated for these species. Unfortunately, there are still a very limited amounts of DNA sequence data available for meaningful comparison in the case of many bark and ambrosia beetles. However, along with morphological characters (**Figures 3 – 22**), the DNA sequence data provided confimation for our identifications of 18 species i.e. *Crossotarsus externedentatus, Euplatypus parallelus, Cryphalus mangiferae, Dryocoetes uniseriatus, Eccoptopterus spinosus, Hypothenemus eruditus, Hypothenemus seriatus, Hylastes angustatus, Styracoptinus murex, Orthotomicus erosus, Scolytoplatypus fasciatus, Strombophorus capensis, Premnobius cavipennis, Xyleborus sexesenii, Xyleborus affinis, Xyleborus ferrugineus, Xyleborus madagascariensis,* and *Xylosandrus crassiusculus.*

4.5. Catalogue

The following section includes a list of all species that have been recorded in South Africa. Names of species not present in the catalogue by Wood & Bright (1992) are indicated in **bold face**. Type specimens (T) are indicated. The collections in which type specimens for species are deposited are indicated and collection numbers given where available. Species known only to be present in South Africa from published literature are indicated (P). Provincial collection data from within the country is given where available.

Species names are based on their most current taxonomic classification. Various taxonomic amendments to species names were considered and included. These include (i) the large revision of the Platypodinae by Wood (1993), (ii) the introductions of the genera *Amphiscolytus* and *Diuncus* (Mandelshtam & Beaver 2003, Hulcr & Coganto 2009), (iii) the revision of the genus *Xylosandrus* by Dole & Cognato (2010), (iv) the introduction of many new synonymies and combinations in the genus

Hylesinopsis by Beaver et al. (2010), (v) the transfer of *Premnobius* to the Premnobiini tribe by Cognato (2013), (vi) the large revision of the Cryphalini just completed by Johnson et al. (2020), and (vii) minor taxonomic changes introduced by Beaver (1998, 2011) & Alonzo-Zarazaga & Lyal (2009).

Species are arranged first by family, then by tribe, then by genus and all are listed alphabetically. Species reported for the first time were treated individually in the discussion section. Species were numbered to aid in keeping track of each species introduced in the catalogue. Common names of beetles and fungal associates were given where known.

FAMILY PLATYPODINAE

TRIBE Platypodini

1. *Crossotarsus externedentatus* (Fairmaire, 1849)

Common name: Exotic pinhole borer

Specimens housed at: Ditsong Museum, FABI

South African provincial collection records: KwaZulu-Natal, Limpopo.

Associated fungi: *Raffaelea albimanens, R. canadensis, R. hennertii* (Scott & du Toit 1970, Harrington et al. 2010)

2. ***Crossotarsus wallacei*** (Thomsom, 1858)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

3. ***Cylindropalpus auricomans*** (Schaufuss, 1897)

Specimens housed at: SANCI

South African provincial collection records: Western Cape.

4. *Doliopygus bohemani* (Chapuis, 1865)

Specimens housed at: Ditsong Museum

South African provincial collection records: Mpumalanga.

5. *Doliopygus chapuisi* (Duvivier, 1891)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

6. *Doliopygus citri* Schedl, 1948

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: KwaZulu-Natal, Western Cape.

7. *Doliopygus crinitus* (Chapuis, 1865)

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Gauteng, Mpumalanga.

8. *Doliopygus dubius* (Sampson, 1942)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

9. *Doliopygus erichsoni* (Chapuis, 1865) **Figure 3 (A-D)**

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Limpopo, Western Cape.

10. *Doliopygus mimicus* (Schedl, 1933)

Specimens housed at: SANCI

South African provincial collection records: Mpumalanga.

11. *Doliopygus spatiosus* (Schedl, 1935)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

12. *Euplatypus hintzi* (Schaufuss, 1897)

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Mpumalanga.

13. *Euplatypus parallelus* (Fabricius, 1801) **Figure 3 (E-H)**

Common name: Ambrosia pinhole borer

Specimens housed at: Ditsong Museum, FABI

South African provincial collection records: KwaZulu-Natal, Limpopo.

14. *Platypus impressus* (Strohmeyer, 1912)

Specimens housed at: Ditsong Museum

South African provincial collection records: KwaZulu-Natal.

15. ***Platypus perrisi*** Chapuis, 1865

Specimens housed at: SANCI

South African provincial collection records: Eastern Cape.

16. *Platypus sampsoni* (Schedl, 1933)

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Mpumalanga, KwaZulu-Natal, Western Cape.

17. *Platypus solutus* Schedl, 1933

Specimens housed at: SANCI

South African provincial collection records: KwaZulu-Natal.

18. *Platypus spinulosus* (Strohmeyer, 1912)

South African provincial collection records: KwaZulu-Natal.

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Schedl (1982) and Wood & Bright (1992).

19. *Triozastus banghaasi* (Schaufuss, 1905)[P]

Specimens housed at SANCI but not of South African origin.

Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

20. *Triozastus marshalli* (Sampson, 1924)[P]

NOTES: No representative specimens of this species from South Africa were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

TRIBE Tesserocerini

21. ***Mitosoma crenulatum*** Chapuis, 1865[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

22. *Mitosoma paulianum* Schedl, 1950[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

23. *Periommatus longicollis* Chapuis 1865

Specimens housed at both the Ditsong Museum and SANCI

South African provincial collection records: KwaZulu-Natal, Mpumalanga.

24. *Periommatus pseudocamerunus* Nunberg, 1958[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

FAMILY SCOLYTINAE

TRIBE Amphiscolytini

1. *Amphiscolytus capensis* (Schedl, 1971) **Figure 4**

Specimens housed at: FABI

South African provincial collection records: KwaZulu-Natal, Limpopo, Western Cape.

TRIBE Cryphalini

2. ***Cryphalus asperatus*** (Gyllenhal, 1813)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

3. *Cryphalus giganteus* Schedl, 1975[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

4. *Cryphalus mangiferae* (Stebbing, 1914) **Figure 5**

Common names: mango bark beetle, shoot gun perforator

Specimens housed at: FABI

South African provincial collection records: Mpumalanga.

Associated fungi: *Ceratocystis manginecans*, *C. magicola*, *C. mangivora* (Van Wyk et al. 2011, Al Adawi et al. 2013).

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Bright (2021).

Commonly known as the mango bark beetle, *C. mangiferae* was described by Stebbing in 1912 in the genus *Hypocryphalus*. Over the last decade, this beetle has been found to vector three pathogenic species of *Ceratocystis* responsible for both mango sudden death disease and mango blight, two diseases that are causing devastating losses to the mango crops of Brazil, Oman, and Pakistan (Van Wyk et al. 2007, 2011, Masood & Saeed 2012, Al Adawi et al. 2013). The taxonomy of these *Ceratocystis* spp.

residing in the *Ceratocystis fimbriata* sensu lato complex is controversial, but the aggressiveness of these fungi is well recognised (Oliveira et al. 2015, Kanzi et al. 2020). Given that South Africa produces approximately 75 000 tons of mango annually (SAMGA 2020), the detection of *C. mangiferae* warrants closer investigation. As the fungi vectored by the beetle has not yet been found in South Africa, *C. mangiferae* is regarded as a potential threat to the South African mango growing industry (Galdino et al. 2016).

5. ***Cryphalus margadilaonis*** (Hopkins, 1915)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

6. ***Cryphalus rotundus*** (Hopkins, 1915)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

TRIBE Crypturgini

7. ***Aphanarthum capense*** Jordal, 2009[P]

South African provincial collection records: Eastern Cape

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Jordal (2009).

8. ***Apanarthum maculatum*** Jordal, 2009[P]

South African provincial collection records: Eastern Cape

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Jordal (2009).

9. ***Coleobothrus germeauxi*** Menier, 1973

Specimens housed at: SANCI

South African provincial collection records: Limpopo.

TRIBE Diamerini

10. ***Diamerus hispidus*** (Klug, 1832)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

11. ***Diamerus pulverulentus*** Gertaecker, 1873

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: KwaZulu-Natal, Limpopo.

12. ***Pernophorus abhorrens*** Eggers, 1943

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Mpumalanga.

13. *Pernophorus pondoanus* Eggers, 1933[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Schedl (1962) & Wood & Bright (1992).

South African provincial collection records: Eastern Cape.

14. *Pseudodiamerus obscurus* Eggers, 1943[P]

NOTES: No representative specimens of this species from South Africa were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

15. ***Pseudodiamerus striatus*** Eggers, 1933[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

16. ***Sphaerotrypes expressus*** Schedl, 1937

Specimens housed at: SANCI

South African provincial collection records: Mpumalanga.

17. ***Sphaerotrypes hagedorni*** Eggers, 1920

Specimens housed at: SANCI

South African provincial collection records: Eastern Cape.

NOTES: Zwolinksi & Geldenhuis (1988) detected this species infesting ironwood in South Africa. This record was not included in the catalogue by Wood & Bright (1992).

18. ***Sphaerotrypes pila*** Blandford, 1894[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

19. *Sphaerotrypes pygeumi* Schedl, 1955

Specimens housed at: SANCI

South African provincial collection records: Limpopo.

NOTES: *Sphaerotrypes pyguemi* was first reported to be present in South Africa according by Browne (1970).

20. ***Sphaerotrypes variegatus* Eggers, 1932**

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Cape Province (Old designation), Limpopo.

21. *Strombophorus capensis* Schedl, 1965[T] **Figure 6**

Holotype: SANCI (TYPH00598)

Specimens housed at: Ditsong Museum, SANCI, FABI

South African provincial collection records: Eastern Cape, Gauteng, Kwazulu-Natal, Limpopo, Western Cape.

22. ***Strombophorus crenatus*** Hagedorn, 1909[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

23. *Strombophorus ericius* (Schaufuss, 1897)

Specimens housed at: Ditsong Museum

South African provincial collection records: Kwazulu-Natal.

TRIBE Diapodini

24. ***Diapus africanus*** Beaver, 2000[P]

South African provincial collection records: Eastern Cape.

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Bright (2014).

TRIBE Dryocoetini

25. *Coccotrypes carpophagus* (Hornung, 1842)

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Gauteng, KwaZulu-Natal, Western Cape.

26. ***Coccotrypes confusus*** (Eggers, 1940)

Specimens housed at: Ditsong Museum

South African provincial collection records: KwaZulu-Natal.

27. *Coccotrypes congonus* Eggers, 1924

Specimens housed at: Ditsong Museum

South African provincial collection records: KwaZulu-Natal.

28. *Coccotrypes dactyliperda* (Fabricius, 1801)

Common names: Date stone beetle, palm seed borer, button beetle.

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Eastern Cape, Gauteng, KwaZulu-Natal.

29. ***Coccotrypes niger*** Eggers, 1927[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Bright (2021).

30. ***Cyrtogenius africus*** Wood, 1988

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Limpopo.

NOTES: *Cyrtogenius africus* was reported to be present in South Africa by Van Der Linde et al. (2016) infesting dying *Euphorbia ingens*.

Associated fungi: *Ophiostoma thermarum*, *Aureovirgo volanis* (Van Der Linde et al. 2016).

31. ***Cyrtogenius bicolor*** Strohmeyer, 1910[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

32. *Cyrtogenius major* Strohmeyer 1911

Specimens housed at: SANCI

South African provincial collection records: Gauteng.

33. *Cyrtogenius silvaniae* Schedl, 1982[T]

Holotype: Ditsong Museum

Specimens housed at: Ditsong Museum

South African provincial collection records: Mpumalanga.

34. ***Dryocoetes uniseriatis*** **Eggers, 1926 Figure 7**

Specimens housed at: FABI

South African provincial collection records: KwaZulu-Natal.

HOST: *Pinus* sp.

35. *Thamnurgus capensis* Schedl, 1977[P]

NOTES: No representative specimens of this species available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

36. ***Thamnurgus euphorbiae*** (Kuester, 1845)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

37. *Thamnurgus euryopsis* Schedl, 1955

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: KwaZulu-Natal.

38. *Thamnurgus lobeliae* Eggers, 1933[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

39. *Thamnurgus longipilis* Schedl, 1938[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

40. *Thamnurgus senecionis* Schedl, 1938[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

41. *Triotemnus striatus* Eggers, 1936

Specimens housed at: Ditsong Museum

South African provincial collection records: KwaZulu-Natal.

42. ***Triotemnus subretusus*** Wollaston, 1864[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

TRIBE Ernoporini

43. ***Eidophelus afer*** (Schedl, 1970)

Specimens housed at: SANCI

South African provincial collection records: Mpumalanga.

NOTES: Schedl (1975) reported the species under its synonym *Cryphalophilus afer* from South Africa. This report was not included in the catalogue by Wood & Bright (1992).

44. *Eidophelus africanus* (Schedl, 1977)[P]

South African provincial collection records: KwaZulu-Natal.

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

45. *Eidophelus aspericollis* (Eichhoff, 1878)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

46. ***Eidophelus darwinii*** (Eichhoff, 1878)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

47. *Eidophelus devius* (Schedl, 1975)

Specimens housed at: Ditsong Museum

South African provincial collection records: Gauteng, Mpumalanga.

48. *Eidophelus grobleri* (Schedl, 1962) [T]

Holotype: SANCI (TYPH00547)

Specimens housed at: SANCI

South African provincial collection records: Western Cape.

49. *Eidophelus onyanganus* (Schedl, 1941) **Figure 8**

Specimens housed at: FABI

South African provincial collection records: Limpopo, Mpumalanga.

50. ***Eidophelus spessivtzevi*** Berger, 1917[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

51. *Eidophelus spirostachius* (Schedl, 1958)

Specimens housed at: SANCI

South African provincial collection records: KwaZulu-Natal, Mpumalanga.

52. ***Eidophelus usagaricus*** (Eggers, 1922)[N]

Specimens housed at: SANCI

South African provincial collection records: Western Cape.

53. *Ernoporus parvulus* (Eggers, 1943)

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Northern Cape.

54. ***Hypothenemus acaciae*** (Eggers, 1920)

Specimens housed at: Ditsong Museum

South African provincial collection records: Gauteng.

55. *Hypothenemus africanus* (Hopkins, 1915)[P]

South African provincial collection records: Western Cape.

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

56. *Hypothenemus agnatus* (Eggers, 1924)

Specimens housed at: Ditsong Museum

South African provincial collection records: Gauteng.

57. *Hypothenemus areccae* (Hornung, 1842)

Specimens housed at: SANCI

South African provincial collection records: Gauteng.

58. ***Hypothenemus birmanus*** (Eichhoff, 1878)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Bright (2021).

59. *Hypothenemus biseriatus* (Eggers, 1919)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

60. *Hypothenemus brevicollis* (Eggers, 1927)

Specimens housed at: Ditsong Museum

South African provincial collection records: Mpumalanga.

61. *Hypothenemus carbonarius* (Eggers, 1943)

Specimens housed at: SANCI

South African provincial collection records: Gauteng, Limpopo, Northern Cape.

62. *Hypothenemus crudiae* (Panzer, 1791) **Figure 9 (A-B)**

Specimens housed at: FABI

South African provincial collection records: Limpopo.

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

63. ***Hypothenemus dexter*** **(Sampson 1922)**

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Mpumalanga.

NOTES: Schedl (1957) reported the species under its synonym *Cryphalus dexter* from South Africa. This report was not included in the catalogue by Wood & Bright (1992).

64. *Hypothenemus elephas* (Eichhoff, 1872)

Specimens housed at: SANCI

South African provincial collection records: KwaZulu-Natal.

65. *Hypothenemus eruditus* (Westwood, 1834) **Figure 9 (C-D)**

Common name: Erudite pygmy beetle

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Eastern Cape, Gauteng, Limpopo, Mpumalanga, Western Cape.

66. *Hypothenemus hampei* (Ferrari, 1867)

Common names: Coffee borer beetle, Coffee berry borer.

Specimens housed at: SANCI

South African provincial collection records: Limpopo.

67. ***Hypothenemus liberiensis*** (Hopkins, 1915)

Specimens housed at: Ditsong Museum

South African provincial collection records: Mpumalanga.

68. *Hypothenemus mallyi* (Hopkins, 1915)

Specimens housed at: Ditsong Museum

South African provincial collection records: Gauteng.

69. *Hypothenemus malus* (Schedl 1957)

Specimens housed at: SANCI

South African provincial collection records: Gauteng.

70. *Hypothenemus marshalli* (Eggers, 1936)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

71. *Hypothenemus morio* (Eggers, 1940)

Specimens housed at: Ditsong Museum

72. *Hypothenemus mozambiquensis* Eggers, 1943

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Limpopo.

73. *Hypothenemus natalensis* (Schedl, 1941)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

74. *Hypothenemus ruginosus* Wood, 1989

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

75. *Hypothenemus seriatus* (Eichhoff, 1872) **Figure 9 (E-F)**

Specimens housed at: Ditsong Museum, SANCI, FABI

South African provincial collection records: KwaZulu-Natal, Limpopo, Mpumalanga, Northern Cape, Western Cape.

76. *Hypothenemus setosus* (Eichhoff, 1868) **Figure 9 (G-H)**

Specimens housed at: Ditsong Museum, FABI

South African provincial collection records: Limpopo.

77. *Hypothenemus solitarius* (Schedl, 1950)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

78. *Hypothenemus vitis* (Browne, 1970)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

TRIBE Hexacolini

79. *Microborus boops* Blandfors, 1897

Specimens housed at: Ditsong Museum

TRIBE Hylastini

80. *Hylastes angustatus* (Herbst, 1793) **Figure 10**

Specimens housed at: Ditsong Museum, SANCI, FABI

South African provincial collection records: Limpopo, Kwazulu-Natal, Mpumalanga, Western Cape.

Associated fungi: *Ophiostoma ips*, *O. pluriannulatum*, *O. stenoceras*, *Leptographium lundbergii*, *L. serpens*, *Ceratocystiopsis minuta* (Zhou et al. 2001).

81. ***Hylastes ater*** (Paykull, 1800)[P]

Common name: Black pine bark beetle.

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

Associated fungi: *Leptographium procerum, Ophiostoma galeiformis, O. huntii, O. floccosum, O. setosum, O quercu,* (Reay et al. 2005).

82. *Hylastes linearis* Erichson, 1836

Specimens housed at: SANCI

South African provincial collection records: Western Cape.

83. ***Hylurgops palliatus*** (Gyllenhal, 1813)

Common name: Lesser spruce bark beetle.

Specimens housed at: SANCI

Associated fungi: *Leptographium lundbergii, L. guttulatum, L. wingfieldii, Ophiostoma ips, O. minus, O. olivaceum, O. picea, O. pluriannulatum, O. quercus, O. stenoceras* (Jankowiak 2006, Romon et al. 2007)

84. ***Hylurgops rugipennis pinifex*** (Fitch, 1858)

Specimens housed at: SANCI

TRIBE Hylesinini

85. *Dactylipalpus cicatricosus* (Blandford, 1896)

Specimens housed at: Ditsong Museum

South African provincial collection records: KwaZulu-Natal.

86. *Dactylipalpus grouvellei* (Blandford, 1896)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Schedl (1960) & Wood & Bright (1992).

South African provincial collection records: KwaZulu-Natal.

87. ***Dactylipalpus transversus*** Chapuis, 1869[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

88. *Hapalogenius africanus* (Eggers, 1933)

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Mpumalanga, Limpopo.

89. *Hapalogenius fuscipennis* (Chapuis, 1869) **Figure 11**

Specimens housed at: Ditsong Museum, SANCI, FABI

South African provincial collection records: Eastern Cape, Free State, Limpopo.

90. ***Hapalogenius globosus*** Hagedorn, 1912[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

91. ***Hapalogenius pusillus*** (Gerstacker, 1855)

South African provincial collection records: Western Cape.

NOTES: No representative specimens of this species were available in the collections visited. *Hylesinopsis pusillus* was reported to be present in South Africa by Beaver et al. (2010).

92. *Hylesinopsis ericius* (Schaufuss, 1897)

South African provincial collection records: KwaZulu-Natal.

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

93. ***Hylesinopsis fasciata*** (Hagedorn, 1909)

Specimens housed at: Ditsong Museum

South African provincial collection records: KwaZulu-Natal.

NOTES: *Hylesinopsis fasciata* was reported to be present in South Africa by Beaver et al. (2010).

94. ***Hylesinopsis orientalis*** (Eggers, 1943)

Specimens housed at: Ditsong Museum

South African provincial collection records: Limpopo.

95. ***Hylesinopsis sulcatus*** (Eggers, 1944)

Specimens housed at: Ditsong Museum

South African provincial collection records: Mpumalanga.

NOTES: *Hylesinopsis sulcatus* was reported to be present in South Africa by Beaver et al. (2010).

96. ***Hylesinopsis togonus*** (Eggers, 1919)

Specimens housed at: SANCI

South African provincial collection records: Mpumalanga

97. *Hylesinus aculeatus* Say, 1926

Common name: Eastern ash bark beetle

Specimens housed at: SANCI

South African provincial collection records: Eastern Cape.

98. *Rhopalopselion bimaculatus* (Eggers, 1933)[P]

South African provincial collection records: KwaZulu-Natal.

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

99. ***Rhopalopselion bituberculatum*** Hagedorn, 1909[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

TRIBE Hylurgini

100. *Hylurgus ligniperda* (Fabricius, 1787)

Common name: red-haired pine bark beetle

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Western Cape.

Associated fungi: *Ceratocystiopsis minuta, Grosmannia olivacea, Leptographium huntii, L. lundbergii, L. tereforme. L. truncatum, L. serpens, Ophiostoma bicolor, O. canum, O. flocossum, O. galeiformis, O. ips. O. picea, O. pluriannulatum, O. quercus, O. rectangulosprium, O. stenoceras* (Zhou et al. 2001, Kim et al. 2011, Jankowiak & Bilanski 2013, Davydenki et al. 2014)

101. *Xylechinus australis* Schedl, 1957

Specimens housed at: Ditsong Museum

South African provincial collection records: Mpumalanga.

102. ***Xylechinus pilosus*** (Ratzeburg, 1837)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

TRIBE Hypoborini

103. ***Dacryostactus kolbei*** Schaufuss, 1905 **Figure 12 (A-B)**

Specimens housed at: SANCI, FABI

South African provincial collection records: Gauteng, KwaZulu-Natal.

NOTES: Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

104. *Glochiphorus alienus* Schedl, 1982[T]

Holotype: Ditsong Museum

Specimens housed at: Ditsong Museum

South African provincial collection records: Gauteng.

105. ***Glochiphorus globosus*** Strohmeyer, 1910[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

106. *Liparthrum australis* Schedl, 1975[T]

Holotype: Ditsong Museum

Specimens housed at: Ditsong Museum

South African provincial collection records: Mpumalanga.

107. ***Liparthrum bituberculatum*** Wollaston, 1854[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

108. *Styracoptinus euphorbiae* (Bright, 1981)

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992)

109. *Styracoptinus murex* (Blandford, 1896) **Figure 12 (C-D)**

Specimens housed at: Ditsong Museum, SANCI, FABI

South African provincial collection records: Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga.

TRIBE Ipini

110. ***Acanthotomicus biconicus*** (Schedl, 1938)[N]

Specimens housed at: Ditsong Museum

111. ***Acanthotomicus lefevrei*** (Browne, 1973)[N]

Specimens housed at: SANCI

South African provincial collection records: Mpumalanga.

112. *Acanthotomicus medius* (Eggers, 1943)

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

113. *Acanthotomicus pilosus* (Eggers, 1924)

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

114. ***Acanthotomicus spinosus*** Blandford, 1894[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

115. ***Ips typographus*** (Linnaeus, 1758)[P]

Common name: European spruce bark beetle.

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

Associated fungi: *Ceratocystiopsis minuta, Ceratocystis polonica, Graphium pseudormiticum, Grosmannia penicillate, G. europhioides, Ophiostoma ainoae, O. bicolor, O. cucullata, O. europhioides, O. japonicum, O. penicillatum, O. picea* (Yamaoka et al. 1997, Persson et al. 2009, Zhao et al. 2019).

116. *Orthotomicus caelatus* (Eichhoff, 1868)

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Western Cape.

117. *Orthotomicus erosus* (Wollaston, 1857) **Figure 13 (A-B)**

Common name: Pine bark beetle

Specimens housed at: SANCI, FABI

South African provincial collection records: Kwazulu-Natal, Western Cape.

Associated fungi: *Ophiostoma ips, O. pluriannulatum, Leptographium lundbergii, L. serpens* (Zhou et al. 2001)

118. ***Orthotomicus laricis*** (Fabricius, 1793)[P]

Common name: Lesser larch bark beetle.

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

Associated fungi: *Ophiostoma ips, O. peregrinum, O. piliferum* (De Errasti et al. 2016)

119. *Pityokteines curvidens* (Germar, 1824)[P]

Common name: Fir engraver beetle.

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

TRIBE Micracidini

120. *Afromicracis convexus* (Schedl, 1962)

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

121. ***Afromicracis kenyaensis*** Schedl, 1959[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

122. *Afromicracis natalensis* (Eggers, 1936)

Specimens housed at: Ditsong Museum

123. *Lanurgus barbatus* Eggers, 1920

Specimens housed at: SANCI

South African provincial collection records: Gauteng.

124. *Lanurgus capensis* Schedl, 1965[T]

Holotype: SANCI (TYPH00556)

Specimens housed at: SANCI

South African provincial collection records: Eastern Cape.

125. *Lanurgus gracilis* Schedl, 1958[T]

Holotype: Ditsong Museum

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Eastern Cape.

126. *Lanurgus podocarpi* Schedl, 1955 **Figure 14 (A-B)**

Specimens housed at: Ditsong Museum, SANCI, FABI

South African provincial collection records: Eastern Cape, KwaZulu-Natal.

NOTES: *Lanurgus podocarpi* and *Lanurgus bicolor* were synonymized by Beaver (2011). The holotype specimen of *Lanurgus bicolor* is located at SANCI (TYPH00550).

127. *Lanurgus rhusi* Schedl, 1962[T]

Holotype: SANCI (TYPH00536)

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Gauteng.

128. *Lanurgus spathulatus* Schedl, 1948

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Gauteng, KwaZulu-Natal, Eastern Cape.

129. *Lanurgus subsulcatus* Browne, 1970[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

130. *Lanurgus widdringtoniae* Schedl, 1962[T]

Holotype: SANCI (TYPH00552)

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Western Cape.

131. *Lanurgus xylographus* Schedl, 1962[T] **Figure 14 (C-D)**

Holotype: SANCI (TYPH00553)

Specimens housed at: Ditsong Museum, SANCI, FABI

South African provincial collection records: Gauteng, KwaZulu-Natal, Western Cape.

NOTES: *Lanurgus xylographus* and *Lanurgus oleaeformis* were synonymized by Beaver (2011). The holotype specimen of *Lanurgus oleaeformis* is located at SANCI (TYPH00551).

132. ***Phloeocurus africanus*** (Schedl, 1957)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

133. ***Pseudomicracis africanus*** Schedl, 1957

Specimens housed at: SANCI

South African provincial collection records: Limpopo.

NOTES: Schedl (1975) reported the species under its synonym *Hylocurus africanus* from South Africa. This report was not included in the catalogue by Wood & Bright (1992).

134. *Traglostus brevisetosus* Schedl, 1957

South African provincial collection records: KwaZulu-Natal.

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Schedl (1957) and Wood & Bright (1992).

135. ***Traglostus exornatus*** Schedl, 1938[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

136. *Traglostus longipilis* Schedl, 1958[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

137. *Traglostus pubescens* Schedl, 1982[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

TRIBE Phloeosinini

138. *Cladoctonus affinis* Strohmeyer, 1911

Specimens housed at: Ditsong Museum

South African provincial collection records: KwaZulu-Natal, Limpopo.

139. *Cladoctonus eggersi* Wichmann, 1911

Specimens housed at: SANCI

South African provincial collection records: Mpumalanga.

140. *Cladoctonus natalensis* Eggers, 1936

Specimens housed at: Ditsong Museum

South African provincial collection records: Eastern Cape, KwaZulu Natal.

141. ***Phloeosinus armatus*** Reitter, 1887 **Figure 15**

Common name: Cypress shoot beetle.

Specimens housed at: FABI

South African provincial collection records: Gauteng.

TRIBE Polygraphini

142. *Polygraphus basutoae* Schedl, 1957

Specimens housed at: SANCI

South African provincial collection records: Eastern Cape, Western Cape.

143. *Polygraphus natalensis* Eggers, 1919 **Figure 16**

Specimens housed at: Ditsong Museum, SANCI, FABI

South African provincial collection records: Eastern Cape, Free State, Gauteng.

144. *Polygraphus poligraphus* (Linnaeus, 1758)

Common names: Small spruce bark beetle.

South African provincial collection records: Eastern Cape.

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Schedl (1957) and Wood & Bright (1992).

Associated fungi: *Endoconidiophora polonica* (Kronoke & Solheim 1997)

145. ***Polygraphus pubescens*** (Fabricius, 1793)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

146. *Polygraphus rufipennis* (Kirby, 1837)

Common name: Four eyed spruce bark beetle.

Specimens housed at: SANCI

South African provincial collection records: Eastern Cape.

Associated fungi: *Grosmannia abietina* (Oshawa et al. 2000).

TRIBE Premnobiini

147. *Premnobius cavipennis* Eichhoff, 1878 **Figure 13 (C-D)**

Specimens housed at: Ditsong Museum, SANCI, FABI

South African provincial collection records: Gauteng, KwaZulu-Natal, Limpopo.

Associated fungi: *Affroraffaelea ambrosiae* (Bateman et al. 2017).

148. *Premnobius declivis* Eggers, 1944[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

149. *Premnobius nodulosus* Hagedorn, 1908[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

150. *Premnobius spinifer* Eggers, 1927

Specimens housed at: Ditsong Museum

South African provincial collection records: Mpumalanga.

TRIBE Scolytini

151. *Scolytus kirchi* Skalitzky, 1999[P]

Common name: Elm bark beetle.

South African provincial collection records: Eastern Cape (Stellenbosch).

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Six et al. (2005).

Associated fungi: *Ophiostoma ulmi*, *O novo-ulmi*.

TRIBE Scolytoplatypodini

152. *Scolytoplatypus fasciatus* Hagedorn, 1904 **Figure 17**

Specimens housed at: Ditsong Museum, SANCI, FABI

South African provincial collection records: Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, Western Cape.

Associated fungi: *Wolfgangiella franznegeri* (Mayers et al 2020)

153. *Scolytoplatypus obtectus* Schedl, 1975[T]

Holotype: SANCI (TYPH00555)

Specimens housed at: SANCI

South African provincial collection records: Eastern Cape.

154. ***Scolytoplatypus occidentalis***

Specimens housed at: SANCI

South African provincial collection records: Western Cape.

NOTES: Zwolinksi & Geldenhuis (1988) detected this species infesting Cape beech in South Africa. This record was not included in the catalogue by Wood & Bright (1992).

155. ***Scolytoplatypus opacicollis*** Eggers, 1936

Specimens housed at: SANCI

South African provincial collection records: Western Cape.

NOTES: *Scolytoplatypus opacicollis* is a species of ambrosia beetle described by Eggers in 1936. The species has been recorded from a single African country, Zambia (Wood & Bright 1992).

156. ***Scolytoplatypus permirus*** (Schaufuss, 1891)

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

Associated fungi: *Wolfgangiella madagascariensis* (Mayers et al. 2020)

TRIBE Trypophloeini

157. *Afrocosmoderes caplandicus* (Schedl, 1965)[T]

Holotype: SANCI (TYPH00548)

Specimens housed at: SANCI

South African provincial collection records: Eastern Cape, Western Cape.

158. *Afrocosmoderes grobleri* (Schedl, 1961)[T]

Holotype: SANCI (TYPH00554)

Specimens housed at: SANCI

South African provincial collection records: Western Cape.

159. *Macrocryphalus elongatus* (Schedl, 1965)

Specimens housed at: SANCI

South African provincial collection records: KwaZulu-Natal.

TRIBE Xyleborini

160. *Ambrosiodmus eichhoffii* (Schreiner, 1882)

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Gauteng, Mpumalanga, Western Cape.

161. *Ambrosiodmus inoblitus* (Schedl, 1970)

Specimens housed at: SANCI

South African provincial collection records: Cape Provice (Old designation), KwaZulu-Natal.

162. *Ambrosiodmus natalensis* (Schaufuss, 1891)

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: KwaZulu-Natal.

163. *Ambrosiodmus obliquus* (LeConte, 1878)

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: Eastern Cape, Limpopo.

164. *Ambrosiodmus opacithorax* (Schedl, 1937)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

165. ***Ambrosiodmus pithecolobius*** (Schedl, 1937) **Figure 18 (C-D)**

Specimens housed at: Ditsong Museum, SANCI, FABI

South African provincial collection records: KwaZulu-Natal, Limpopo.

166. *Ambrosiodmus rhodesianus* (Eggers, 1936)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

167. ***Ambrosiodmus tachygraphus*** (Zimmerman, 1868) **Figure 18 (A-B)**

Specimens housed at: FABI

South African provincial collection records: Limpopo.

NOTES: Alonso-Zarazaga & Lyal (2009) reported this species from South Africa.

168. *Ambrosiodmus tropicus* (Hagedorn, 1910)

Specimens housed at: Ditsong Museum

South African provincial collection records: Eastern Cape.

169. ***Coptoborus adjunctus*** (Eggers, 1924)

Specimens housed at: Ditsong Museum

South African provincial collection records: Mpumalanga

170. *Cyclorhipidion capensis* (Eggers, 1944)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

171. *Cyclorhipidion crucifer* (Hagedorn, 1908)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

172. *Cyclorhipidion neocrucifer* (Schedl, 1955)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

173. ***Cyclorhipidion pelliculosum*** Hagedorn, 1912[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

174. ***Diuncus haberkorni*** (Eggers, 1920) **Figure 18 (E-F)**

Specimens housed at: FABI

South African provincial collection records: Mpumalanga.

HOST: *Macadamia* sp.

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Bright (2021).

Diuncus haberkorni is a South East Asian species of ambrosia beetle described by Eggers in 1920 originally in the genus *Xyleborus*. The genus *Diuncus* was erected in by Hulcr & Cognato (2009) to accommodate 17 species of Xyleborine ambrosia beetle previously treated in *Xyleborus*, including *X. haberkorni*. These species exploit the fungus gardens of other ambrosia beetles in a process known as "crop theft" instead of cultivating their own gardens within their galleries (Hulcr & Cognato 2009, 2010). In the supplementary catalogue by Bright (2021), no distribution or host data for the species is presented relating to its occurrence in South Africa. However, in this study, specimens of the species

were found colonizing Macadamia logs originally from Mpumalanga in association with *Xylosandrus crassiusculus.*

175. ***Eccoptopterus sexspinosus*** Motschulsky, 1863[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

176. *Eccoptopterus spinosus* (Olivier, 1795) **Figure 18 (G-H)**

Specimens housed at: SANCI, FABI

South African provincial collection records: KwaZulu-Natal, Limpopo.

Associated fungi: *Ambrosiella catenulate* (Lin et al. 2017).

177. ***Euwallacea fornicatus*** (Eichhoff, 1868) **Figure 19 (A-B)**

Common name: Polyphagous Shot Hole Borer.

Specimens housed at: FABI

South African provincial collection records: Present in all provinces except Limpopo.

NOTES: *Euwallacea fonicatus* was reported present in South Africa by Paap et al. (2018) infesting *Platanus* sp. Although widespread in the country, no specimens of the species have been deposited in the museum collections visited.

Associated fungi: *Fusarium euwallacea, Graphium euwallacea, Paracremonium pembeum* (Freeman et al. 2013, Lynch et al. 2016).

178. *Euwallacea piceus* (Motschulsky, 1863)

Specimens housed at: Ditsong Museum

South African provincial collection records: Eastern Cape, KwaZulu-Natal.

179. ***Euwallacea similis*** (Ferrari, 1867)

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: KwaZulu-Natal, Mpumalanga.

180. ***Euwallacea wallacei*** (Blandford, 1896)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

181. *Euwallacea xanthopus* (Eichhoff, 1868) **Figure 19 (C-D)**

Specimens housed at: Ditsong Museum, SANCI, FABI

South African provincial collection records: KwaZulu-Natal, Limpopo.

182. *Xyleborinus aemulus* (Wollaston, 1869) **Figure 19 (E-F)**

Specimens housed at: Ditsong Museum, SANCI, FABI

South African provincial collection records: Eastern Cape, KwaZulu-Natal, Limpopo, Western Cape.

Associated fungi: *Sporothrix aemulophila* (Musvuugwa et al. 2015)

183. *Xyleborinus alienus* (Schedl, 1977)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

184. ***Xyleborinus collarti*** (Eggers, 1932)

Specimens housed at: SANCI

South African provincial collection records: KwaZulu-Natal.

185. *Xyleborinus forficulus* (Eggers, 1922)

Specimens housed at: Ditsong Museum

South African provincial collection records: Gauteng.

186. *Xyleborinus pseudopityogenes* (Eggers, 1943)

Specimens housed at: Ditsong Museum

187. *Xyleborinus quadrispinosus* (Eichhoff, 1878)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

188. *Xyleborinus saxesenii* (Ratzeburg, 1837) **Figure 19 (G-H)**

Common name: Fruit tree pinhole borer.

Specimens housed at: Ditsong Museum, SANCI, FABI

South African provincial collection records: Eastern Cape, Limpopo, Western Cape.

Associated fungi: *Raffaelea promiscua, R. sulphurea* (Batra 1967, Nel et al. 2021).

189. *Xyleborinus scleroacryae* (Schedl, 1962)[T]

Holotype: SANCI (TYPH00690)

Specimens housed at: SANCI

South African provincial collection records: KwaZulu-Natal.

190. ***Xyleborinus similans*** (Eggers, 1940)

Specimens housed at: SANCI

South African provincial collection records: KwaZulu-Natal.

191. *Xyleborinus spinifer* (Eggers, 1920)

Specimens housed at: Ditsong Museum

South African provincial collection records: Limpopo.

192. *Xyleborus affinis* (Eichhoff, 1868) **Figure 20 (A-B)**

Common name: Sugarcane Shot Hole Borer.

Specimens housed at: Ditsong Museum, SANCI, FABI

South African provincial collection records: Eastern Cape, Gauteng, KwaZulu-Natal, Limpopo.

Associated fungi: *Raffaelea arxii, R. lauricola, R. subfusca* (Saucedo-Carabez et al. 2018)

193. *Xyleborus alluaudi* Shcaufuss, 1897[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

194. *Xyleborus antaisaka* Schedl, 1953 **Figure 20 (C-D)**

Specimens housed at: Ditsong Museum, SANCI, FABI

South African provincial collection records: KwaZulu-Natal, Limpopo.

195. *Xyleborus barumbuensis* Eggers, 1924[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

196. *Xyleborus conradti* Hagedorn, 1910

Specimens housed at: SANCI

South African provincial collection records: KwaZulu-Natal.

197. *Xyleborus eichhoffianus* Schedl, 1950[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

198. ***Xyleborus elongatus* Eggers, 1920**

Specimens housed at: Ditsong Museum

South African provincial collection records: Eastern Cape.

199. *Xyleborus ferrugineus* (Fabricius, 1801) **Figure 20 (E-F)**

Specimens housed at: Ditsong Museum, SANCI, FABI

South African provincial collection records: Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga.

200. *Xyleborus luteus* Schedl, 1937[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

201. ***Xyleborus madagascariensis*** Schaufuss, 1891

Specimens housed at: SANCI

South African provincial collection records: Limpopo, KwaZulu-Natal.

202. ***Xyleborus perdiligens*** Schedl, 1937 **Figure 20 (G-H)**

Specimens housed at: Ditsong Museum, FABI

South African provincial collection records: Limpopo.

203. *Xyleborus perforans* (Wollaston, 1857)

Common name: Island pinhole borer.

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: KwaZulu-Natal.

204. *Xyleborus volvulus* (Fabricius, 1775)

Specimens housed at: Ditsong Museum, SANCI

South African provincial collection records: KwaZulu-Natal.

Associated fungi: *Raffaelea arxii*, *R. rapanea* (Cruz et al. 2019).

205. *Xyleborus xylographus* (Say, 1826)

Specimens housed at: SANCI

South African provincial collection records: KwaZulu-Natal, Western Cape.

206. *Xylosandrus compactus* (Eichhoff, 1875)

Common names: Black twig borer, Black coffee borer, Black coffee twig borer, Teas stem borer.

Specimens housed at: SANCI

South African provincial collection records: KwaZulu-Natal.

Associated fungi: *Ambrosiella xylebori* (Brader 1964)

207. ***Xylosandrus crassiusculus*** **Figure 21 (C-D)**

Common name: Granulate ambrosia beetle.

Specimens housed at: Ditsong Museum, SANCI, FABI

South African provincial collection records: KwaZulu-Natal, Limpopo, Mpumalanga, Northern Cape.

NOTES: *Xylosandrus crassiusculus* was first reported from South Africa by Nel et al. (2020).

Associated fungi: *Ambrosiella roeperi*, *Ceratocystiopsis lunata* (Harrington et al. 2014, Nel et al. 2021)

208. ***Xylosandrus morigeus*** (Blandford, 1894)[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

TRIBE Xyloctonini

209. ***Cryphalomimus striatus*** Eggers, 1927

Specimens housed at: Ditsong Museum

South African provincial collection records: Limpopo.

210. ***Ctonoxylon auratum*** Hagedorn, 1910[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

211. *Ctonoxylon bosqueiae* Schedl, 1962

Specimens housed at: Ditsong Museum

South African provincial collection records: Northern Cape.

212. *Ctonoxylon griseum* Schedl, 1941

Specimens housed at: SANCI

South African provincial collection records: Western Cape.

213. *Ctonoxylon hamatum* Schedl, 1941

Specimens housed at: SANCI

South African provincial collection records: Western Cape.

214. ***Ctonoxylon longipilum*** Eggers, 1935

Specimens housed at: Ditsong Museum

215. *Ctonoxylon methneri* Eggersi, 122

Specimens housed at: Ditsong Museum

216. *Ctonoxylon uniseriatum* Schedl, 1965[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992) and Beaver (2011).

217. ***Glostatus declividepressus*** Schedl, 1939[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Alonso-Zarazaga & Lyal (2009).

218. *Glostatus delicatus* (Schedl, 1962)

Specimens housed at: SANCI

South African provincial collection records: Eastern Cape, Western Cape.

219. *Glostatus pondoanus* Schedl, 1958[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992) and Beaver (2011)

220. *Glostatus squamosus* Schedl, 1962 **Figure 21 (A-B)**

Specimens housed at: SANCI, FABI

South African provincial collection records: KwaZulu-Natal, Limpopo, Western Cape.

221. *Stephanopodius dispar* (Eggers, 1936)

Specimens housed at: Ditsong Museum

222. *Stephanopodius dubiosus* (Schedl, 1970)[T]

Holotype: SANCI (TYPH00549)

Specimens housed at: SANCI

South African provincial collection records: Western Cape.

223. *Stephanopodius giganteus* Schedl, 1950[P]

NOTES: No representative specimens of this species were available in the collections visited. Records to indicate the presence of this species in South Africa are presented in Wood & Bright (1992).

224. *Xyloctonus latus* Eggers, 1939

Specimens housed at: SANCI

South African provincial collection records: Western Cape.

225. *Xyloctonus maculatus* Schedl, 1965*[T]

Holotype: SANCI (TYPH00557)

Specimens housed at: SANCI

South African provincial collection records: Eastern Cape, Western Cape.

226. *Xyloctonus pubifer* Schedl, 1965

Specimens housed at: Ditsong Museum

227. *Xyloctonus scolytoides* Eichhoff, 1872 **Figure 21 (C-D)**

Specimens housed at: Ditsong Museum, FABI

South African provincial collection records: Eastern Cape, Gauteng, Limpopo.

4.5.1. Dubious records

4.5.1.1. The locality information for the following species located in the Ditsong museum needs to be verified. If confirmed they represent first reports from South Africa.

228. ***Scolytus intricatus*** (Ratzeburg, 1837)

Specimens housed at: Ditsong Museum

NOTES: *Scolytus intricatus*, commonly known as the European oak bark beetle, is a species of bark beetle described by Ratzeburg, originally in the genus *Eccoptogaster*. Prior to this study the species has only been reported from two North African countries, Morocco, and Tunisia (Wood & Bright 1992). Native to Europe, this species is a common pest of oak (*Quercus* sp.) and some other hardwood tree species (Doganlar & Schopf 1984).

229. ***Scolytus mali*** (Beehstain, 1805)

Specimens housed at: Ditsong Museum

NOTES: *Scolytus mali*, commonly known as the larger shot hole borer or large fruit bark beetle, is a species of bark beetle described by Beehstain, originally in the genus *Bostrichus*. Prior to this study the species has only been reported from three North African countries, Algeria, Egypt, and Morocco (Wood & Bright 1992). Likely native to Europe, the species has been spread to various regions including large parts of North America where it infests and colonizes fruit trees such as apple (*Malus* spp.) and cherry (*Prunus* spp.) species (Smith & Cognato, 2014).

230. ***Scolytus ratzeburgi*** Janson, 1856

Specimens housed at: Ditsong Museum

NOTES: *Scolytus ratzeburgi*, commonly known as the birch bark beetle or birch sapwood borer, is a species of bark beetle described by Janson in 1856. Prior to this study the species had not previously been reported from Africa (Wood & Bright 1992). As its common name suggests, this species is a common inhabitant of Birch (*Betula* spp.) in various parts of Europe (Wood & Bright 1992, Lukić et al. 2019).

231. ***Scolytus rugulosus*** (Muller, 1818)

Specimens housed at: Ditsong Museum

NOTES: *Scolytus rugulosus*, commonly known as the small fruit bark beetle, is a species of bark beetle described by Muller originally in the genus *Bostrichus*. Prior to this study the species has only been reported from three North African countries, Algeria, Morocco, and Tunisia (Wood & Bright 1992). Native to Europe, the species has been spread to various regions including North America where it infests and colonizes stone and pome fruit trees (Smith & Cognato, 2014).

232. ***Scolytus scolytus*** (Fabricius, 1775)

Specimens housed at: Ditsong Museum

NOTES: *Scolytus scolytus*, commonly known as the larger European elm bark beetle or just elm bark beetle, is a species of bark beetle described by Fabricius originally in the genus *Bostrichus*. Prior to this study the species had not previously been reported from Africa. Native to Europe this species can be found primarily infesting elm (*Ulmus* spp.) (Wood & Bright 1992) and has been found to be a potential vector of the Dutch elm disease pathogen *Ophiostoma ulmi* (Webber 2004).

4.5.1.2. The description of the following species is questionable and needs to be reconsidered.

233. ***Hapalogenius tuberculifer*** Mandelshtam, 2006[T]

Holotype: Ditsong Museum

Specimens housed at: Ditsong Museum

South African provincial collection records: Eastern Cape.

NOTES: A holotype of the proposed species is available from the Ditsong Museum but not record of the species ever being published can be found.

234. ***Cryphalomimus maculatus*** Mandelshtam, 2006[T]

Holotype: Ditsong Museum

Specimens housed at: Ditsong Museum

South African provincial collection records: Limpopo.

NOTES: A holotype of the proposed species is available from the Ditsong Museum but not record of the species ever being published can be found.

235. ***Dendrophthorus capensis***[P]

NOTES: The genus *Dendrophthorus* was introduced into the Scolytinae by Dejean in 1835 to accommodate the single species *D. capensis*. However, the description was based on a single specimen and Alonso-Zarazaga & Lyal (2009) ruled the true species identity as unknown.

4.5.1.3. The identification of the following species is uncertain and needs to be confirmed. If confirmed it will represent a first report from South Africa.

236. Cladoctonus amanicus

Specimens housed at: Ditsong Museum

South African provincial collection records: Limpopo.

NOTES: *Cladoctonus amanicus* is a species of bark beetle described by Eggers in 1920. Prior to this study this species was only known from Central Africa where it is likely to be native. *Cladoctonus amanicus* is known to infest a single host species *Acacia xanthophloea* (Wood & Bright 1992).

5. Discussion

In the present study, we report 260 bark and ambrosia beetle species occurring in South Africa, this is represented by 24 species of Platypodinae and 236 species of Scolytinae. Of the 97 additional beetle species included in this new checklist, compared to the catalogue produced by Wood & Bright (1992), 24 species are reported from the country for the first time. For the remaining 73 species that were excluded from the Wood & Bright (1992) catalogue, the species were either reported after the publication of the catalogue or published before the catalogue but were not included (**Table 2 footnotes**).

Field surveys and samples submitted by the public, which were incorporated here, accounted for more than 2300 bark and ambrosia beetle specimens. From these, 40 species were identified: three Platypodinae and 38 Scolytinae. Four of these, i.e. *Ambrosiodmus pithecolobius*, *Dryocoetes uniseriatus*, *Phloeosinus armatus*, and *Xyleborus perdiligens* represent first records for South Africa. Due to inconsistencies in the methods of collection over time, conclusive conclusions cannot be made from the field collection data, however overall, the Afromontane collections yielded more species the grassland collections. The difference in host diversity in these regions, in which Afromontane region is more diverse in flora, could explain the higher numbers of beetle species collected from this region.

Over the years many checklists and catalogues recording the species of bark and ambrosia beetles have been published for many countries. Most recently these include catalogues for Cuba (Gomez et al. 2020), parts of North America (Gomez et al. 2018), Ecuador (Martinez et al. 2019), American Samoa (Rabgalia *et al* 2020), parts of Russia (Mandelshtam & Selikhovkin 2020), Brazil (Sandoval Rodriguez et al. 2017) and more and some of these have resulted in the identification and description of new species. This just serves to emphasize the continued importance of diversity surveys as a scientific resource.

5.1. DNA sequencing of bark and ambrosia beetle

In entomological taxonomy there is paucity in moving towards DNA sequence-based identification and many taxonomic studies still rely solely on morphological data. This is largely due to the difficulty in DNA amplification and sequencing of the "universal" markers and due to very high levels of sequence variability (Cognato 2006, Jordal & Coganto 2014, Cognato et al. 2020, Dr. Andrew Johnson pers. comm.). This has resulted in DNA sequence databased being sparsely populated except for well-known, wide-spread, and problematic species. The sequence data generated for 38 different species of collected and identified bark and ambrosia beetle specimens in this study will contribute to filling in the data gap, especially found for African species.

The move towards DNA sequence-based identification does not come without challenges. For instance, fungal taxonomists face a variety of challenges as databases are flooded with DNA sequence data of mis-identified and incorrectly classified species (Nilsson et al. 2006, Raja et al. 2017, Meiklejohn et al. 2019). This introduces an additional challenge in determining the correct taxonomic assignment. Thus, care should be taken with all DNA sequences generated and deposited to ensure correctness within reference sequence databases and to aid, instead of hinder, future research studies (Cognato et al. 2020).

5.2. First reports for South Africa

In this study we collected 24 species that, to the best of our knowledge, represent first reports for South Africa. Specimens for these species have either been collected during this study (four) or representative specimens were available in the entomological collections visited (20). The dubious records are not included in this section and information for these species was presented earlier in the text.

5.2.1. Species originating from Eurasia

Platypus perrisi is an Asian ambrosia beetle described in 1865 by Chapuis. So far it appears that this beetle has only been reported to infest a single host species, *Shorrea robusta* (Wood & Bright 1992).

Dryocoetes uniseraitus is an Asian bark beetle described by Eggers in 1926 (Wood & Bright 1992, Shimizu et al. 2013). An unidentified *Dryocoetes* specimen is available at South African National Collection of Insects, ARC Biosystematics Division, Pretoria, South Africa, originating from KwaZulu-Natal. This specimen matches in morphology to one we collected and sequenced from Swaziland that was identified as *Dryocoetes uniseriatis*. Specimens from the African National Collection of Insects date back to the early 1940s where they were discovered in logs imported from Georgia, USA. Although little work is available on *D. uniseriatus*, it is known to preferentially infest Japanese red and black pine (*Pinus densiflora* and *P. thunbergii*) (Shimizu et al. 2013). Recent surges in the number of *D. uniseriatus*, along with those of *Orthotomicus erosus*, infesting *P. patula*, *P. elliottii* and *P. elliottii* x *caribeae* plantations in Swaziland have been noticed. Trees in the South African highveld into Swaziland have been severely stressed in the past few years due to the decline in annual rainfall likely resulting in these surges in bark beetle populations. This is very concerning as extended drought periods are becoming more and more common in South Africa, and continued population increases in these insects could result in large economic losses.

5.2.2. Species originating from Africa

Acanthotomicus biconicus is a species of bark beetle described by Schedl in 1938 originally in the genus *Myeloborus*. Prior to this study, this species was only known from Central Africa where it is likely to be native. Three species have been reported as host of *A. biconicus*, *Albizzia gummifera*, *Millettia* sp. and *Motandra guinecnsis* (Wood & Bright 1992).

Acanthotomicus lefevrei is a species of bark beetle described by Browne in 1973 originally in the genus *Mimips*. Thus far this species has only been recorded in a single African country, the Democratic republic of Congo. The host range and biology of this species is not currently known.

Ambrosiodmus pithecolobius is a species of ambrosia beetle described by Schedl in 1937 originally in the genus *Xyleborus*. Prior to this study, this species was only known from Central Africa where it is likely to be native. *Ambrosiodmus pithecolobius* is only known to infest a single host, *Pithecolobium saman* (Wood & Bright 1992).

Coptoborus adjunctus is an African species of Scolytine ambrosia beetle. The species has been reported from numerous Afrotropical countries, reaching as far south as Zimbabwe. The species has only reported infesting a single host species, *Albizzia gummifera* (Wood & Bright 1992).

Ctonoxylon longipilum is a species of bark beetle described by Eggers in 1957. In 1998, Beaver synonymized *C. longipilum* and *C. nodosum*, recognizing *C. nodosum* as the male of *C. longipilum.* Prior to this study, this species was only known from Central Africa where it is likely to be native (Wood & Bright 1992).

Cylindropalpus auricomans is an African ambrosia beetle species described by Schaufuss in 1897. Although polyphagous, this species preferably infests tree species in the genera of the Caesalpiniaceae (Beaver et al. 1985).

Eidophelus usagaricus is a species of bark beetle described by Eggers in 1922 originally described in the genus *Neocryphalus*. Johnson et al. (2020) recently revised the taxonomy of this species and transferred it to the genus *Eidophelus.* Before the publication of this study this species was only known from Central Africa where it is likely to be native (Wood & Bright 1992).

Hypothenemus liberiensis is a species of bark beetle described by Hopkins in 1915 in the genus *Stephanoderes*. Prior to this study this species was only known from Central Africa where it is likely to be native. *Hypothenemus liberiensis* is known to infest *Theoboroma cacao*, *Coffea* sp. and some flowering Fabaceae (Wood & Bright, 1992).

Hylesinopsis orientalis is an African species of bark beetle described by Eggers is 1943. Thus far the species is only known from two Central African countries, Kenya and Uganda, where it is likely to be native (Wood & Bright 1992).

Scolytoplatypus opacicollis is a species of ambrosia beetle described by Eggers in 1936. Prior to this study, this species has been recorded from a single African country, Zambia (Wood & Bright 1992).

Xyleborinus collarti is a species of ambrosia beetle described by Eggers in 1932 originally in the genus *Xyleborus*. Prior to this study, this species was only known from Central Africa where it is likely to be native. *Xyleborinus collarti* is known to infest at least 10 different host species including species in the genus *Acacia* which are grown commercially in some parts of South Africa (Wood & Bright 1992).

Xyleborinus similans is a species of ambrosia beetle described by Eggers in 1940 originally in the genus *Xyleborus*. Prior to this study, this species was only known from Central Africa where it is likely to be native. Although little research is available on *X. similans*, it is likely to be polyphagous, having been reported to infest at least 17 different species (Wood & Bright 1992). However, reportedly infestation only occurs in felled trees (Browne 1963).

Xyleborus elongatus is a species of ambrosia beetle described by Eggers in 1920. Prior to this study, this species was only known from Central Africa where it is likely to be native. The species is known to infest two host species, *Drypetes leonensis* and *Maba abyssinica* (Wood & Bright 1992).

Xyleborus perdiligens is a species of ambrosia beetle described by Schedl in 1937. Prior to this study, this species was only known from Central Africa where it is likely to be native (Wood & Bright 1992).

5.2.3. Species originating from North America

Hylurgops rugipennis pinifex is a North American species of bark beetle described by Fitch in 1858 originally in the genus *Hylastes*. Like *Hylurgops palliatus, H. rugipennis pinifex* is not considered a pest species in its native range, but preferentially infests species of Pinaceae (Wood & Bright 1992) some of which are grown commercially in South Africa.

5.2.4. Species originating from the Mediterranean

Phloeosinus armatus, commonly known as the cypress bark beetle, is an East Mediterranean bark beetle species described by Reitter in 1887. This beetle primarily infests *Cupresses sempervirens* (or Mediterranean cypress). In recent years, *P. armatus* has been disseminated to various countries including Israel, Italy, and the USA where it has established and become a serious pest on various

species of cypress, as well as *Juniperus* and *Thuja* spp. (Haack 2006, Mendel 1984, Pennacchio et al, 2013). Although cypress is not grown commercially in South Africa, it is a common ornamental species found in gardens country wide.

6. Conclusions

The checklist of bark and ambrosia beetles produced in this study shows clearly that South Africa is home to a rich biodiversity of these insects. Many of the newly recorded species in this study are likely native to Africa as they have only been recorded from countries on the continent. However, some species such as *Diuncus haberkorni, Dryocoetes uniseriatus, Euwallacea fornicatus, Hypothenemus areccea, Xylosandrus crassiusculus,* and *Platypus perrisi* have been introduced from Asia where they are known to be native. These introductions highlight South Africa's vulnerability and lack of policy to effectively manage the introduction of bark and ambrosia beetles This study should provide a foundation on which to base informed and effective quarantine policy in future.

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Table 1. Summary of Platypodinae ambrosia beetle species from South Africa

Tribe	Genus	Species	Schedl	Wood & Bright	Other	Museum specimens from SA	Specimens collected during this study	First report from South Africa	Continental Distribution	Sequenced in this study
Platypodini	*Crossotarsus*	*externedentatus*	x	x		x	x		Africa, Asia, Australia, New Zealand	x
	Crossotarsus	*wallacei*			1				Africa, Asia, Australia, New Zealand	
	Cylindropalpus	*auricomans*				x		x	Africa	
	Doliopygus	*bohemani*	x	x		x			Africa	
	Doliopygus	*chapuisi*		x					Africa	
	Doliopygus	*citri*		x		x			Africa	
	Doliopygus	*crinitus*	x	x		x			Africa	
	Doliopygus	*dubius*	x	x					Africa	
	Doliopygus	*erichsoni*	x	x		x	x		Africa	
	Doliopygus	*mimicus*		x		x			Africa	
	Doliopygus	*spatiosus*		x		x			Africa	
	Euplatypus	*hintzi*	x	x		x			Africa	
	Euplatypus	*parallelus*		x		x	x		Africa, North America, South America	x
	Platypus	*impressus*		x		x			Africa	
	Platypus	*perrisi*				x		x	Asia	
	Platypus	*sampsoni*	x	x		x			Africa*	
	Platypus	*solutus*	x	x		x			Africa	
	Platypus	*spinulosus*	x	x					Africa	
	Triozastus	*banghaasi*		x		x			Africa	
	Triozastus	*marshalli*		x					Africa	
Tesserocerini	*Mitosoma*	*paulianum*	x	x					Africa	
	Mitosoma	*crenulatum*			1				Africa	
	Periommatus	*longicollis*	x	x		x			Africa	
	Periommatus	*pseudocamerunus*		x					Africa	

* Only recorded from South Africa on the African continent

1. Alonso-Zarazaga & Lyal (2009)

Table 2. Summary of Scolytinae bark and ambrosia beetle species from South Africa.

Tribe	Genus	Species	Schedl	Wood & Bright	Other Ref.	Museum specimens from SA	Specimens collected during this study	First report from Africa*	Continental Distribution	Sequenced in this study
Amphiscolytini	*Amphiscolytus*	*capensis*		x		x	x		Africa*	x
Cryphalini	*Cryphalus*	*asperatus*			1				Cosmopolitan	
	Cryphalus	*giganteus*		x					Africa*	
	Cryphalus	*mangiferae*			9		x		Africa, Asia, Australia, North America, South America	x
	Cryphalus	*margadilaonis*							Africa	
	Cryphalus	*rotundus*			1				Africa, Asia, Australia, North America, New Zealand, South America	
Crypturgini	*Aphanarthum*	*capense*			10				Africa*	
	Aphanarthum	*maculatum*			10				Africa*	
	Coleobothrus	*germeauxi*			2	x			Africa	
Diamerini	*Diamerus*	*hispidus*			1	x			Africa, Asia, Australia	
	Diamerus	*pulverulentus*	x			x			Africa	
	Pernophorus	*abhorrens*	x			x			Africa	
	Pernophorus	*pondoanus*		x					Africa*	
	Pseudodiamerus	*obscurus*		x					Africa	
	Pseudodiamerus	*striatus*			1				Africa	
	Sphaerotrypes	*expressus*	x			x			Africa	
	Sphaerotrypes	*hagedorni*			3	x			Africa	
	Sphaerotrypes	*pila*			1				Africa, Asia	
	Sphaerotrypes	*pygeumi*		x		x			Africa	
	Sphaerotrypes	*variegatus*	x			x			Africa	
	Strombophorus	*capensis*	x	x		x	x		Africa*	x
	Strombophorus	*crenatus*			1	x			Africa	
	Strombophorus	*ericius*		x		x			Africa	
Diapodini	*Diapus*	*africanus*			11				Africa*	
Dryocoetini	*Coccotrypes*	*carpophagus*	x	x		x			Cosmopolitan	

Tribe	Genus	Species	Schedl	Wood & Bright	Other Ref.	Museum specimens from SA	Specimens collected during this study	First report from Africa*	Continental Distribution	Sequenced in this study
	Coccotrypes	*confusus*	x			x			Africa	
	Coccotrypes	*congonus*		x		x			Africa	
	Coccotrypes	*dactyliperda*	x	x	1	x			Cosmopolitan	
	Coccotrypes	*niger*			9				Africa	
	Cyrtogenius	*africus*			4	x			Africa	
	Cyrtogenius	*bicolor*			1				Africa, Asia, Australia	
	Cyrtogenius	*major*	x	x		x			Africa	
	Cyrtogenius	*silvaniae*	x	x		x			Africa*	
	Dryocoetes	*uniseriatus*				x	x	x	Asia	x
	Thamnurgus	*capensis*	x	x					Africa*	
	Thamnurgus	*euphorbiae*			1				Africa, Europe	
	Thamnurgus	*euryopsis*	x	x		x			Africa*	
	Thamnurgus	*lobeliae*	x	x					Africa	
	Thamnurgus	*longipilis*	x	x					Africa	
	Thamnurgus	*senecionis*	x	x					Africa	
	Triotemnus	*striatus*	x	x		x			Africa*	
	Triotemnus	*subretusus*			1				Africa, Europe	
Ernoporini	*Eidophelus*	*afer*	x	x		x			Africa	
	Eidophelus	*africanus*		x					Africa*	
	Eidophelus	*aspericollis*		x					Africa, Asia, Europe	
	Eidophelus	*darwinii*			1				Africa, Asia, Australia, North America, South America	
	Eidophelus	*devius*	x	x		x			Africa*	
	Eidophelus	*grobleri*	x	x		x			Africa*	
	Eidophelus	*onyanganus*		x		x	x		Africa	x
	Eidophelus	*spessivtzevi*			1				Africa*, Asia, Europe, North America	
	Eidophelus	*spirostachius*	x	x		x			Africa*	
	Eidophelus	*usagaricus*				x		x	Africa	
	Ernoporus	*parvulus*		x		x			Africa	

Tribe	Genus	Species	Schedl	Wood & Bright	Other Ref.	Museum specimens from SA	Specimens collected during this study	First report from Africa*	Continental Distribution	Sequenced in this study
	Hypothenemus	*acaciae*	x			x			Africa	
	Hypothenemus	*africanus*		x					Africa*, Asia, North America, South America	
	Hypothenemus	*agnatus*	x	x		x			Africa	
	Hypothenemus	*areccae*		x		x			Africa, Asia, Europe, North America, South America	
	Hypothenemus	*birmanus*			9				Africa, Micronesia	
	Hypothenemus	*biseriatus*		x					Africa	
	Hypothenemus	*brevicollis*		x		x			Africa	
	Hypothenemus	*carbonarius*	x	x		x			Africa	
	Hypothenemus	*crudiae*		x			x		Africa, Asia, North America, South America	x
	Hypothenemus	*dexter*	x			x			Africa	
	Hypothenemus	*elephas*		x		x			Africa	
	Hypothenemus	*eruditus*	x	x		x	x		Cosmopolitan	x
	Hypothenemus	*hampei*	x	x		x			Africa, Asia, North America, South America	
	Hypothenemus	*liberiensis*				x		x	Africa	
	Hypothenemus	*mallyi*		x		x			Africa	
	Hypothenemus	*malus*		x		x			Africa*	
	Hypothenemus	*marshalli*		x					Africa	
	Hypothenemus	*morio*		x		x			Africa	
	Hypothenemus	*mozambiquensis*		x		x			Africa	
	Hypothenemus	*natalensis*	x	x					Africa*	
	Hypothenemus	*ruginosus*	x	x					Africa*	
	Hypothenemus	*seriatus*	x	x		x	x		Africa, Asia, Australia, North America, South America	x
	Hypothenemus	*setosus*		x		x	x		Africa, Asia, North America, South America	x
	Hypothenemus	*solitarius*	x	x					Africa	
	Hypothenemus	*vitis*		x					Africa*	
Hexacolini	*Microborus*	*boops*		x		x			Africa, North America, South America	

Tribe	Genus	Species	Schedl	Wood & Bright	Other Ref.	Museum specimens from SA	Specimens collected during this study	First report from Africa*	Continental Distribution	Sequenced in this study
Hylastini	*Hylastes*	*angustatus*	x	x		x	x		Africa*, Asia, Europe	x
	Hylastes	*ater*			1				Africa, Asia, Australia, Europe, North America	
	Hylastes	*linearis*	x	x		x			Africa*, Asia, Europe	
	Hylurgops	*palliatus*	x			x			Africa*, Asia, Europe	
	Hylurgops	*rugipennis pinifex*				x		x	Africa*, North America	
Hylesinini	*Dactylipalpus*	*cicatrcosus*	x	x		x			Africa	
	Dactylipalpus	*grouvellei*		x					Africa	
	Dactylipalpus	*transversus*			5				Africa, Asia, Australia	
	Hapalogenius	*africanus*	x	x		x			Africa	
	Hapalogenius	*fuscipennis*	x	x		x	x		Africa	x
	Hapalogenius	*globosus*			1				Africa	
	Hapalogenius	*pusillus*			5				Africa	
	Hylesinopsis	*ericius*		x					Africa*	
	Hylesinopsis	*fasciata*			5	x			Africa	
	Hylesinopsis	*orientalis*			1	x		x	Africa	
	Hylesinopsis	*sulcatus*			5	x			Africa	
	Hylesinopsis	*togosus*	x			x			Africa	
	Hylesinus	*aculeatus*	x			x			Africa*	
	Rhopalopselion	*bimaculatus*		x					Africa*, North America Africa*	
	Rhopalopselion	*bituberculatum*			1				Africa	
Hylurgini	*Hylurgus*	*ligniperda*	x	x	1	x			Africa, Asia, Australia, Europe, New Zealand, South America	
	Xylechinus	*australis*	x	x		x			Africa	
	Xylechinus	*pilosus*			1				Africa*, Asia, Europe	
Hypoborini	*Dacryostactus*	*kolbei*			1	x	x		Africa	x
	Glochiphorus	*alienus*	x	x		x			Africa*	
	Glochiphorus	*globosus*			1				Africa	
	Liparthrum	*australis*	x	x		x			Africa*	

Tribe	Genus	Species	Schedl	Wood & Bright	Other Ref.	Museum specimens from SA	Specimens collected during this study	First report from Africa*	Continental Distribution	Sequenced in this study
	Liparthrum	*bituberculatum*			1				Africa, Asia, Europe, North America	
	Styracoptinus	*euphorbiae*		x					Africa*	
	Styracoptinus	*murex*	x	x		x	x		Africa	x
Ipini	*Acanthotomicus*	*biconicus*				x		x	Africa	
	Acanthotomicus	*lefevrei*				x		x	Africa	
	Acanthotomicus	*medius*		x					Africa	
	Acanthotomicus	*pilosus*		x					Africa	
	Acanthotomicus	*spinosus*			1				Africa, Asia, Australia, South America	
	Ips	*typographus*			1				Africa, Asia, Australia, Europe, North America, South America	
	Orthotomicus	*caelatus*	x	x		x			Africa, North America	
	Orthotomicus	*erosus*		x		x	x		Africa, Asia, Europe, South America	x
	Orthotomicus	*laricis*			1				Africa, Asia, Europe, North America, South America	
	Pityokteines	*curvidens*		x	1				Africa, Asia, Europe, South America	
Micracini	*Afromicracis*	*convexus*		x					Africa*	
	Afromicracis	*kenyaensis*			1				Africa, Australia	
	Afromicracis	*natalensis*		x		x			Africa*	
	Lanurgus	*barbatus*	x	x	1	x			Africa	
	Lanurgus	*capensis*	x	x		x			Africa*	
	Lanurgus	*gracilis*	x	x		x			Africa*	
	Lanurgus	*podocarpi*	x	x		x	x		Africa	x
	Lanurgus	*rhusi*	x	x		x			Africa*	
	Lanurgus	*spathulatus*	x	x		x			Africa*	
	Lanurgus	*subsulcatus*		x					Africa*	
	Lanurgus	*widdringtoniae*		x		x			Africa*	
	Lanurgus	*xylographus*		x		x	x		Africa	x
	Phloeocurus	*africanus*			1				Africa	
	Pseudomicracis	*africanus*	x			x			Africa	

Tribe	Genus	Species	Schedl	Wood & Bright	Other Ref.	Museum specimens from SA	Specimens collected during this study	First report from Africa*	Continental Distribution	Sequenced in this study
	Traglostus	*brevisetosus*	x	x					Africa*	
	Traglostus	*exornatus*			1				Africa	
	Traglostus	*longipilis*		x					Africa*	
	Traglostus	*pubescens*		x					Africa*	
Phloeosinini	*Cladoctonus*	*affinis*	x			x			Africa	
	Cladoctonus	*eggersi*	x			x			Africa	
	Cladoctonus	*natalensis*		x		x			Africa*	
	Phloeosinus	*armatus*					x	x	Africa*, Asia, North America	x
Polygraphini	*Polygraphus*	*basutoae*	x	x		x			Africa*	
	Polygraphus	*natalensis*	x	x		x	x		Africa	x
	Polygraphus	*poligraphus*	x	x					Africa*, Asia, Europe	
	Polygraphus	*pubescens*			1				Africa, Asia, Europe, North America	
	Polygraphus	*rufipennis*	x	x		x			Africa*, North America	
Premnobiini	*Premnobius*	*cavpennis*	x	x	1	x	x		Africa, North America, South America	x
	Premnobius	*declivis*		x					Africa*	
	Premnobius	*nodulosus*		x					Africa	
	Premnobius	*spinifer*		x		x			Africa	
Scolytoplatypo-dini	*Scolytoplatypus*	*fasciatus*	x	x		x	x		Africa	x
	Scolytoplatypus	*obtectus*		x		x			Africa*	
	Scolytoplatypus	*occidentalis*			3	x			Africa	
	Scolytoplatypus	*opacicollis*				x		x	Africa	
	Scolytoplatypus	*permirus*			1				Africa, Asia	
Scolytini	*Scolytus*	*kirchi*			8				Africa*, Europe, Asia	
Trypophloeini	*Afrocosmoderes*	*caplandicus*	x	x		x			Africa*	
	Afrocosmoderes	*grobleri*	x	x		x			Africa*	
	Macrocryphalus	*elongatus*		x		x			Africa*	
Xyleborini	*Ambrosiodmus*	*eichhoffii*		x		x			Africa	

Tribe	Genus	Species	Schedl	Wood & Bright	Other Ref.	Museum specimens from SA	Specimens collected during this study	First report from Africa*	Continental Distribution	Sequenced in this study
	Ambrosiodmus	*inoblitus*	x	x		x			Africa	
	Ambrosiodmus	*natalensis*	x	x		x			Africa	
	Ambrosiodmus	*obliquus*		x		x			Africa, North America, South America	x
	Ambrosiodmus	*opacithorax*		x					Africa	
	Ambrosiodmus	*pithecolobius*				x	x	x	Africa	x
	Ambrosiodmus	*rhodesianus*		x					Africa	
	Ambrosiodmus	*tachygraphus*			1		x		Africa, Asia, Australia, South America	
	Ambrosiodmus	*tropicus*		x					Africa	
	Coptoborus	*adjunctus*				x		x	Africa	
	Cyclorhipidion	*capensis*		x					Africa*	
	Cyclorhipidion	*crucifer*		x					Africa	
	Cyclorhipidion	*neocrucifer*		x					Africa	
	Cyclorhipidion	*pelliculosum*			1				Africa, Asia	
	Diuncus	*haberkorni*			9		x		Africa, Asia	x
	Eccoptopterus	*sexspinosus*			1				Africa, Asia, Australia	
	Eccoptopterus	*spinosus*	x	x		x	x		Africa, Asia	x
	Euwallacea	*fornicatus*			6		x		Africa, Asia, North America	x
	Euwallacea	*piceus*		x		x			Africa, Asia, Australia	
	Euwallacea	*similis*	x			x			Africa, Asia	
	Euwallacea	*wallacei*			1				Africa, Asia, Australia, North America, South America	
	Euwallacea	*xanthopus*	x	x		x	x		Africa, Asia	x
	Xyleborinus	*aemulus*	x	x		x	x		Africa*	x
	Xyleborinus	*alienus*		x					Africa*	
	Xyleborinus	*collarti*				x		x	Africa	
	Xyleborinus	*forficulus*		x		x			Africa	
	Xyleborinus	*pesudopityogenes*		x		x			Africa	
	Xyleborinus	*quadrispinosus*		x					Africa	

Tribe	Genus	Species	Schedl	Wood & Bright	Other Ref.	Museum specimens from SA	Specimens collected during this study	First report from Africa*	Continental Distribution	Sequenced in this study
	Xyleborinus	*saxesenii*	x	x	1	x	x		Cosmopolitan	x
	Xyleborinus	*scleroacryae*		x		x			Africa*	
	Xyleborinus	*similans*				x		x	Africa	
	Xyleborinus	*spinifer*	x	x		x			Africa	
	Xyleborus	*affinis*	x	x		x	x		Africa, Asia, Australia, North America, New Zealand, South America	x
	Xyleborus	*alluandi*		x					Africa	
	Xyleborus	*antaisaka*		x		x	x		Africa	x
	Xyleborus	*barunbuensis*		x					Africa	
	Xyleborus	*conradti*		x		x			Africa	
	Xyleborus	*eichhoffianus*		x					Africa	
	Xyleborus	*elongatus*				x		x	Africa	
	Xyleborus	*ferrugineus*	x	x		x	x		Africa, Australia, North America, South America	x
	Xyleborus	*luteus*		x					Africa	
	Xyleborus	*madagascariensis*	x	x		x	x		Africa	x
	Xyleborus	*perdiligens*				x	x	x	Africa	x
	Xyleborus	*perforans*		x		x			Africa, Asia	
	Xyleborus	*volvulus*	x	x		x			Africa, Australia, North America, South America	
	Xyleborus	*xylographus*	x			x			Africa*, North America	
	Xylosandrus	*compactus*		x		x			Africa, Asia, North America, South America	
	Xylosandrus	*crassiusculus*			7	x	x		Cosmopolitan	x
	Xylosandrus	*morigeus*			1				Cosmopolitan	
Xyloctonini	*Cryphalomimus*	*striatus*			1	x			Africa	
	Ctonoxylon	*auratum*			1				Africa	
	Ctonoxylon	*bosqueiae*	x	x		x			Africa	
	Ctonoxylon	*griseum*		x		x			Africa	
	Ctonoxylon	*hamatum*	x	x		x			Africa	

Tribe	Genus	Species	Schedl	Wood & Bright	Other Ref.	Museum specimens from SA	Specimens collected during this study	First report from Africa*	Continental Distribution	Sequenced in this study
	Ctonoxylon	*longipilum*				x		x	Africa	
	Ctonoxylon	*methneri*		x		x			Africa	
	Ctonoxylon	*uniseriatum*		x					Africa	
	Glostatus	*declividepressus*			1				Africa	
	Glostatus	*delicatus*	x	x		x			Africa*	
	Glostatus	*pondoanus*		x					Africa*	
	Glostatus	*squamosus*	x	x		x	x		Africa*	x
	Stephanopodius	*dispar*		x	1	x			Africa	
	Stephanopodius	*dubiosus*	x	x		x			Africa*	
	Stephanopodius	*giganteus*		x					Africa	
	Xyloctonus	*latus*	x	x		x			Africa	
	Xyloctonus	*maculatus*	x	x		x	x		Africa*	x
	Xyloctonus	*pubifer*		x		x			Africa	
	Xyloctonus	*scolytoides*	x	x	1	x	x		Africa	
Uncertain Records	*Cladoctonus*	*amanicus*				x		x	Africa	
	Cryphalomimus	*maculatus*				x			Africa*	
	Dendrophthorus	*capensis*			1				Africa*	
	Hapalogenius	*tubeculifer*							Africa*	
	Scolytus	*intricatus*				x		x	Africa, Asia, Europe	
	Scolytus	*mali*				x		x	Africa, Asia, Europe, North America	
	Scolytus	*ratzeburgi*				x		x	Africa*, Asia, Europe	
	Scolytus	*rugulosus*				x		x	Africa, Asia, Europe, North America, South America	
	Scolytus	*scolytus*				x		x	Africa*, Asia, Europe	

*Only recorded from South Africa on the African continent

1. Alonso-Zarazaga & Lyal (2009)
2. Van Der Linde et al. (2018)
3. Zwolinksi & Geldenhuis (1988)
4. Van Der Linde et al. (2016)
5. Beaver et al. (2010)

6. Paap et al. (2018)
7. Nel et al. (2020)
8. Six et al. (2005)
9. Bright (2021)
10. Jordal (2009)
11. Bright (2014)

Table 3. Bark and ambrosia beetle species identified from samples submitted to the FABI Diagnostic clinic

Genus	Species	Province	Collection method	Hosts
Crossotarsus	*externedentatus*	Kwazulu-Natal	Emergence chambers	Macadamia
Dacryostactus	*kolbei*	KwaZulu Natal	Emergence chambers	Unknown
Diuncus	*haberkorni*	KwaZulu Natal	Emergence chambers	Macadamia
Dryocoetes	*uniseriatus*	KwaZulu Natal	Emergence chambers	Pinus sp.
Eccoptopterus	*spinosus*	Limpopo	Sticky trap	Avocado orchard
Euplatypus	*paralellus*	Gauteng	Emergence chambers	Macadamia, Euclea crispa
Euwallacea	*fornicatus*	All provinces excluding Limpopo	Various	Various (FABI, 2021)
Hylastes	*angustatus*	KwaZulu Natal, Limpopo, Mpumalanga, Western Cape	Emergence chambers	Pinus sp.
Crypahlus	*mangiferae*	Mpumalanga	Emergence chambers	Mango
Lanurgus	*podocarpi*	Western Cape	Sent in vials	Unknown
Lanurgus	*xylographus*	Gauteng	Sent in vials	Unknown
Orthotomicus	*erosus*	KwaZulu Natal	Emergence chambers	*Pinus* sp.
Phloeosinus	*armatus*	Gauteng	Sent in vials	Cypress
Polygraphus	*natalensis*	KwaZulu Natal	Emergence chambers	Unknown
Premnobius	*cavipennis*	Limpopo	Sticky trap	Avocado orchard
Scolytoplatypus	*fasciatus*	Limpopo	Sticky trap	Avocado orchard
Styracoptinus	*murex*	KwaZulu Natal	Emergence chambers	Unknown
Xyleborinus	*saxesenii*	Limpopo	Sticky trap	Avocado orchard
Xyleborinus	*ferrugineus*	Limpopo	Sticky trap & Emergence chambers	Avocado orchard, *Pinus eliotii*
Xyleborus	*monographus*	KwaZulu Natal	Emergence chambers	Unknown
Xyloctonus	*scolytoides*	Gauteng	Light trap	Light trap
Xylosandrus	*crassiusculus*	Kwazulu-Natal, Limpopo, Northern Cape	Sticky trap & Emergence chambers	Avocado, Macadamia

Table 4. Bark and ambrosia beetle species sequenced in this study and BLAST result of DNA sequences (GenBank accession numbers to be acquired later)

Subfamily	Genus	Species	Sequence length (bp)			Highest BLAST % identity compared to Morphological ID of species		
			COI	28S	CAD	COI	28S	CAD
Platypodinae	*Crossotarsus*	*externedentatus*	*697*	612	-	99.58	100	-
	Euplatypus	*parallelus*	*672*	-	-	99.13	-	-
Scolytinae	*Amphiscolytus*	*capensis*	*666*	450	254	No sequence data available for the genus on GenBank		
	Cryphalus	*mangiferae*	*676*	479	-	99.57	100	-
	Strombophorus	*capensis*	*683*	459	-	94.58	100	-
	Dryocoetes	*uniseriatis*	-	480	373	-	99.58	3
	Hypothenemus	*crudiae*	-	463	469	No sequence data available for the species on GenBank		
	Hypothenemus	*eruditus*	*690*	462	-	100	98.22	-
	Hypothenemus	*seriatus*	-	465	-	-	99.12	-
	Hypothenemus	*setosus*	*663*	477	-	1	97.37	-
	Eidophelus	*onyanganus*	*344*	246	466	1	98.65	98.36
	Hylastes	*angustatus*	*481*	436	465	100	2	3
	Hapalogenius	*fuscipennis*	*687*	440	469	No sequence data available for the species on GenBank		
	Dacryostactus	*kolbei*	*454*	480	-	6	91.12	-
	Styracoptinus	*murex*	*406*	499	-	1	100	-
	Orthotomicus	*erosus*	*387*	474	462	92.63	98.87	99.03
	Lanurgus	*podocarpi*	*520*	490	-	6	98.54	-
	Lanurgus	*xylographus*	*500*	481	300	95.29	99.07	97.67
	Phloeosinus	*armatus*	*697*	471	435	4	2	3
	Polygraphus	*natalensis*	*631*	471	468	96.38	2	3
	Scolytoplatypus	*fasciatus*	*670*	480	474	94.38	99.79	100
	Ambrosiodmus	*tachygraphus*	*681*	496	472	-	99.19	97.17
	Ambrosiodmus	*pithecolobius*	*708*	456	475	No sequence data available for the species on GenBank		
	Diuncus	*harbekorni*	*679*	507	465	89.08	100	99
	Eccoptopterus	*spinosus*	*668*	537	467	84.58	99.81	97.70
	Euwallacea	*fornicatus*	*653*	-	-	99.85	-	-
	Euwallacea	*xanthopus*	*654*	598	360	93.50	89.66	95.01
	Premnobius	*cavipennis*	*674*	466	474	5	100	100
	Xyleborinus	*aemulus*	*665*	-	467	96.14	-	3
	Xyleborinus	*saxesenii*	*682*	478	475	99.39	99.79	100
	Xyleborus	*affinis*	*683*	495	464	1	99.8	98.28
	Xyleborus	*austaisaka*	*583*	-	358	No sequence data available for the species on GenBank		
	Xyleborus	*ferrugineus*	656	491	474	98.44	100	99.58
	Xyleborus	*madagascariensis*	512	512	-	100	-	-

Xyleborus	*perdiligens*	*522*	477	422	No sequence data available for the species on GenBank		
Xylosandrus	*crassiusculus*	*678*	513	469	100	100	99.79
Glostatus	*squamosus*		465		-	2	-
Xyloctonus	*maculatus*	*621*	-	-	98.86	-	-

1 - No COI sequence data available for this species on GenBank
2 - No LSU sequence data available for this species on GenBank
3 - No CAD sequence data available for this species on GenBank
4 - Only a single COI sequence is available to this species on GenBank, but the sequence is of very low quality and contains many Ns.
5 - The sequences available on GenBank were generated with different primers pairs than those used in this study and have little to no overlap with the sequences generated in this study.
6 - A sequence to a single specimen is available to the species on GenBank but it did not show up in BLAST.

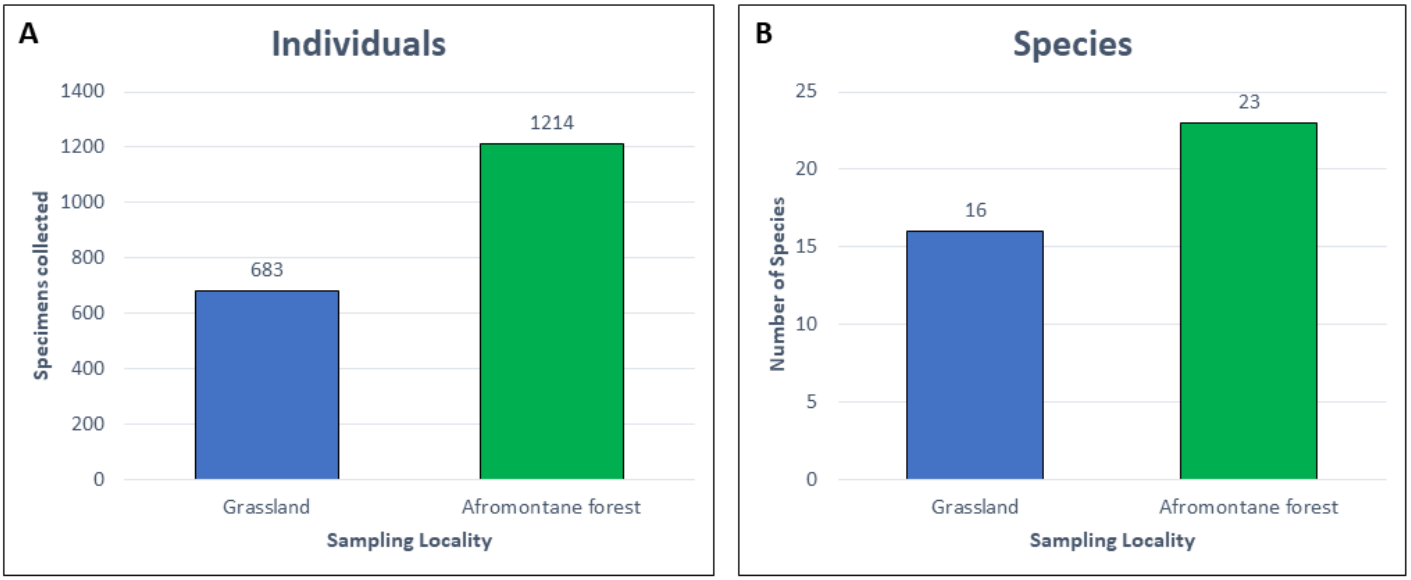

Figure 1. Number of bark and ambrosia beetle specimens collected at the different sampling localities. (A) Number of individual specimens collected at the Grassland and Afromontane forest sampling localities in Tzaneen, Limpopo. (B) Number of individual species collected at the Grassland and Afromontane forest sampling localities in Tzaneen, Limpopo.

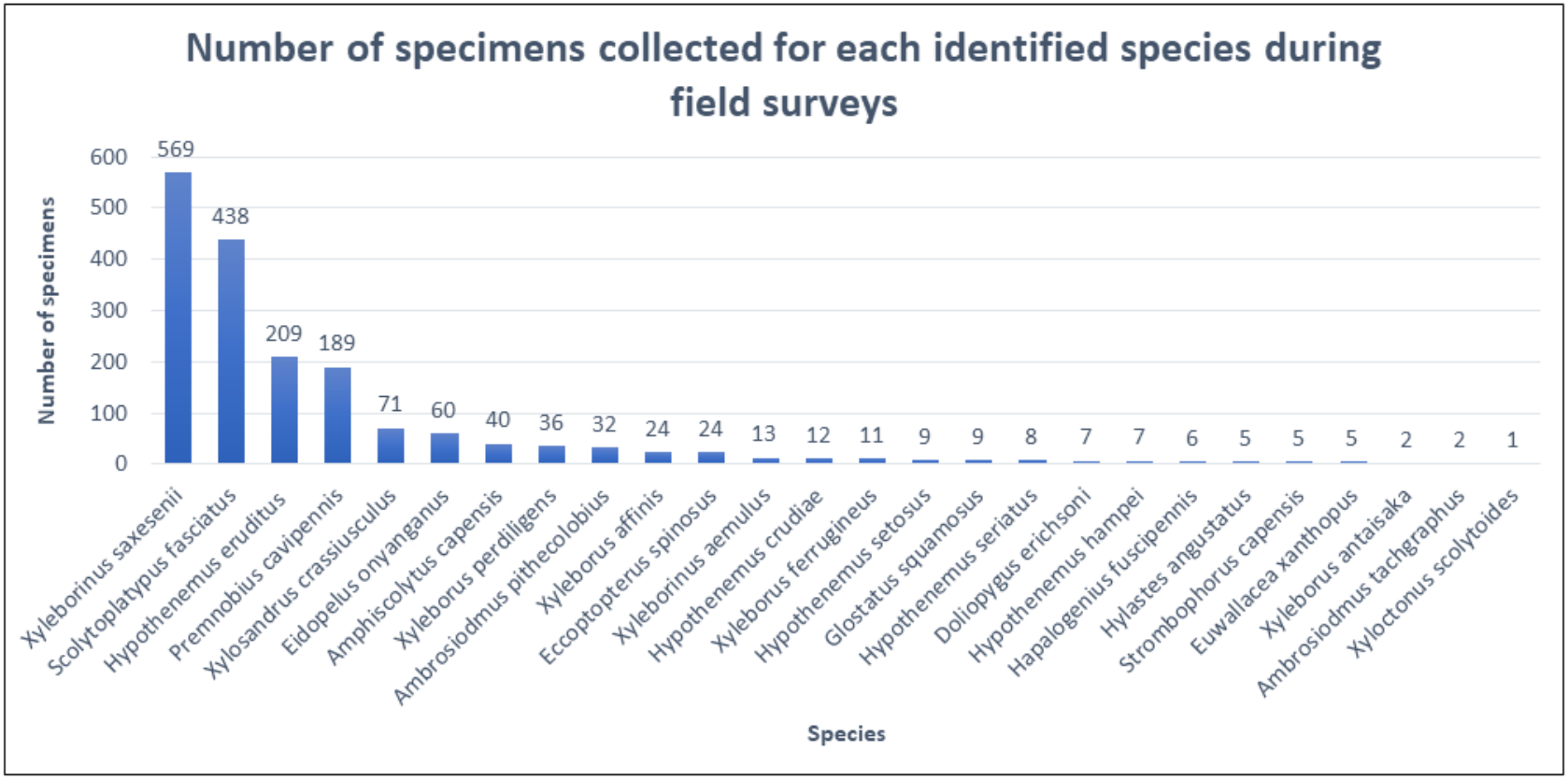

Figure 2. Number of individual specimens collected for each identified species during field surveys carried out in Tzaneen, Limpopo.

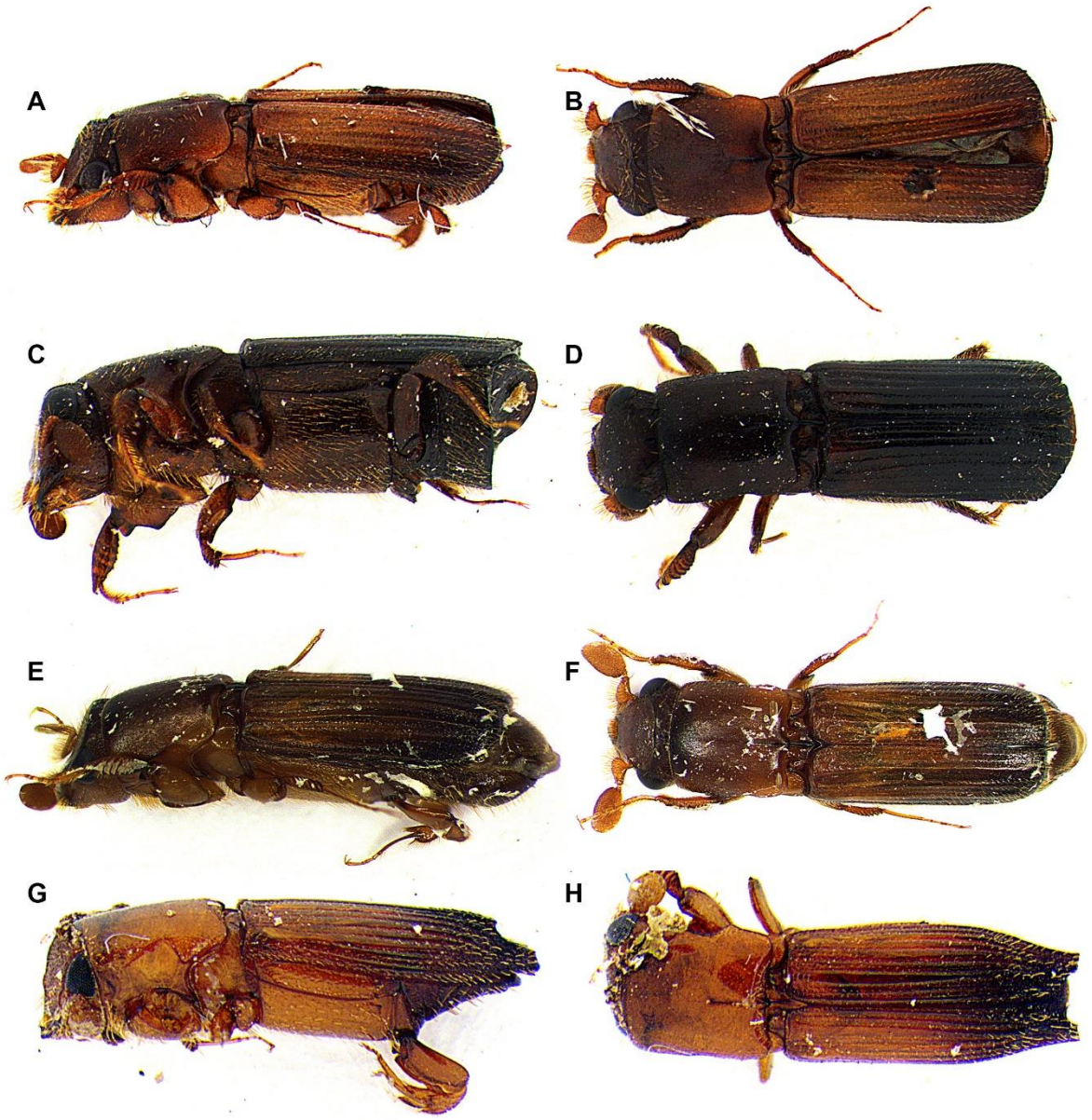

Figure 3. Lateral and dorsal views of Platypodinae species. (A-B) *Doliopygus erichsoni* female, (C-D) *D. erichsoni* male, (E-F) *Euplatypus parallelus* female, (G-H) *E. parallelus* male.

Figure 4. Lateral and dorsal views of *Amphiscolytus capensis.*

Figure 5. Lateral and dorsal views of *Cryphalus mangiferae.*

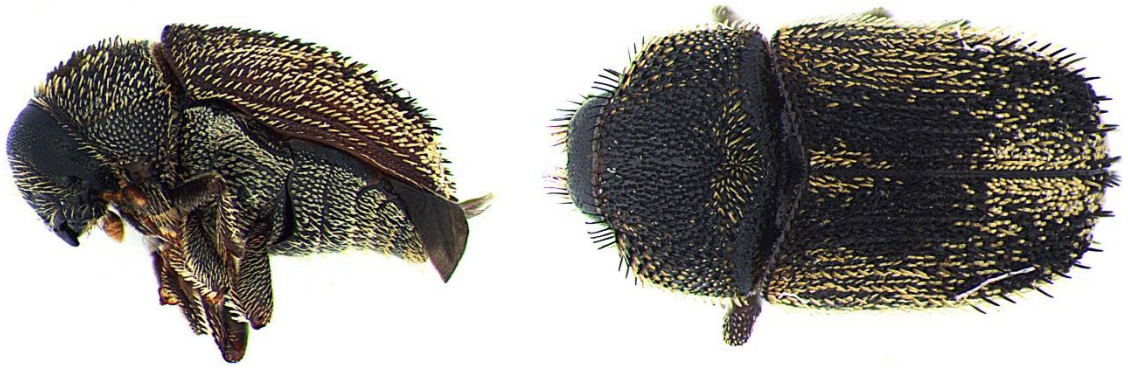

Figure 6. Lateral and dorsal views of *Strombophorus capensis.*

Figure 7. Lateral and dorsal views of *Dryocoetes uniseriatus.*

Figure 8. Lateral and dorsal views of *Hypothenemus* species. (A-B) *Hypothenemus crudiae*, (C-D) *H. eruditus*, (E-F) *H. seriatus*, (G-H) *H. setosus.*

Figure 9. Lateral and dorsal views of *Eidophelus onyanganus*.

Figure 10. Lateral and dorsal views of *Hylastes angustatus*.

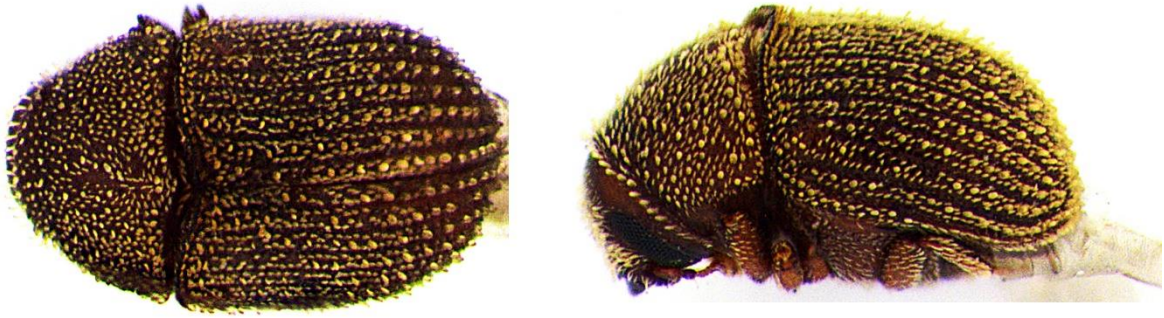

Figure 11. Lateral and dorsal views of *Hapalogenius fuscipennis*

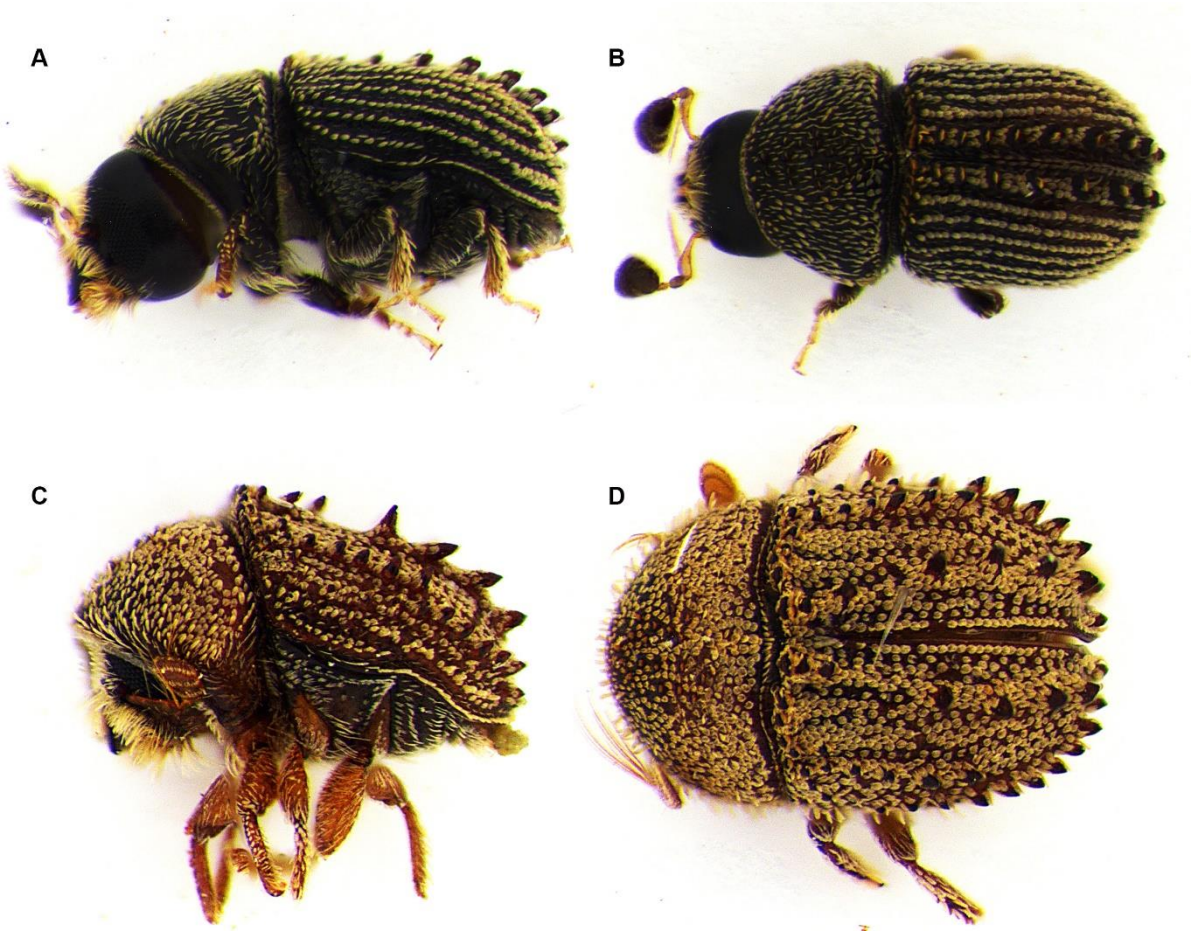

Figure 12. Lateral and dorsal views of Hypoborini. (A-B) *Dacryostactus kolbei*, (C-D) *Styracoptinus murex*.

Figure 13. Lateral and dorsal views of Ipini. (A-B) *Orthotomicus erosus*, (C-D) *Premnobius cavipennis*.

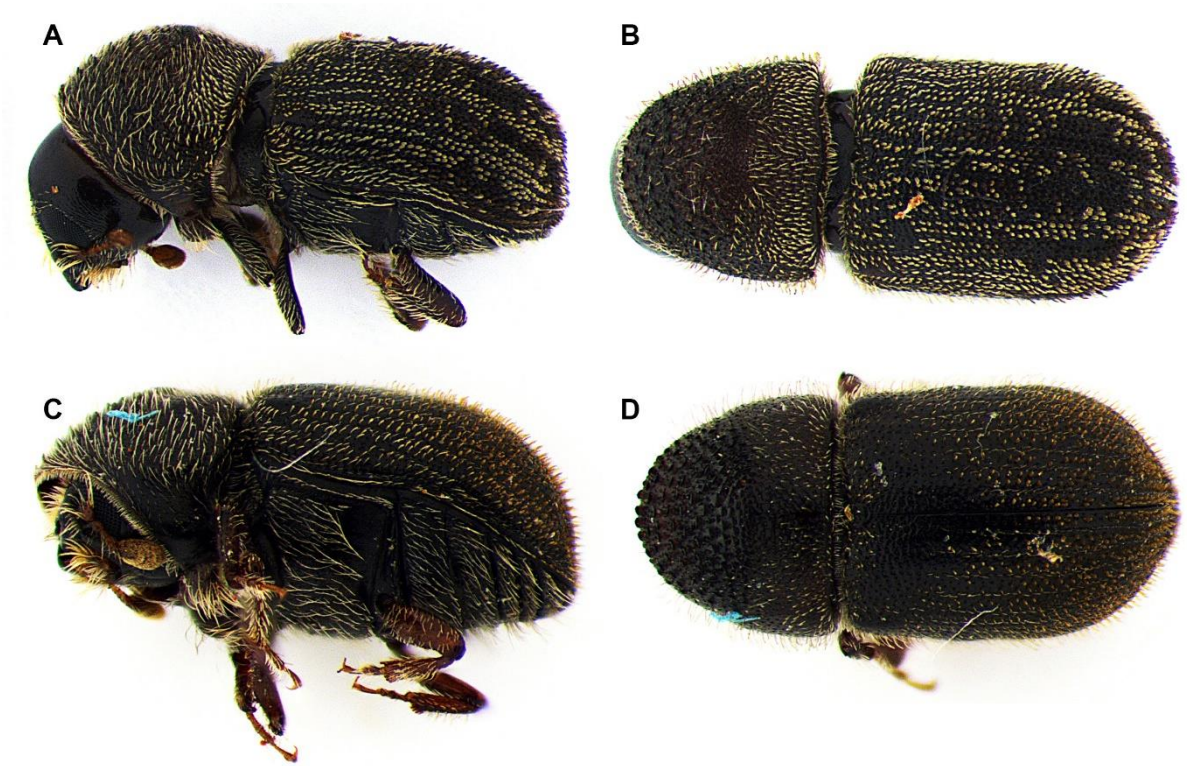

Figure 14. Lateral and dorsal views of Micracidini species. (A-B) *Lanurgus podocarpi*, (C-D) *L. xylographus*

Figure 15. Lateral and dorsal views of *Phloeosinus armatus*.

Figure 16. Lateral and dorsal views of *Polygraphus natalensis*.

Figure 17. Lateral and dorsal views of *Scolytoplatypus fasciatus*.

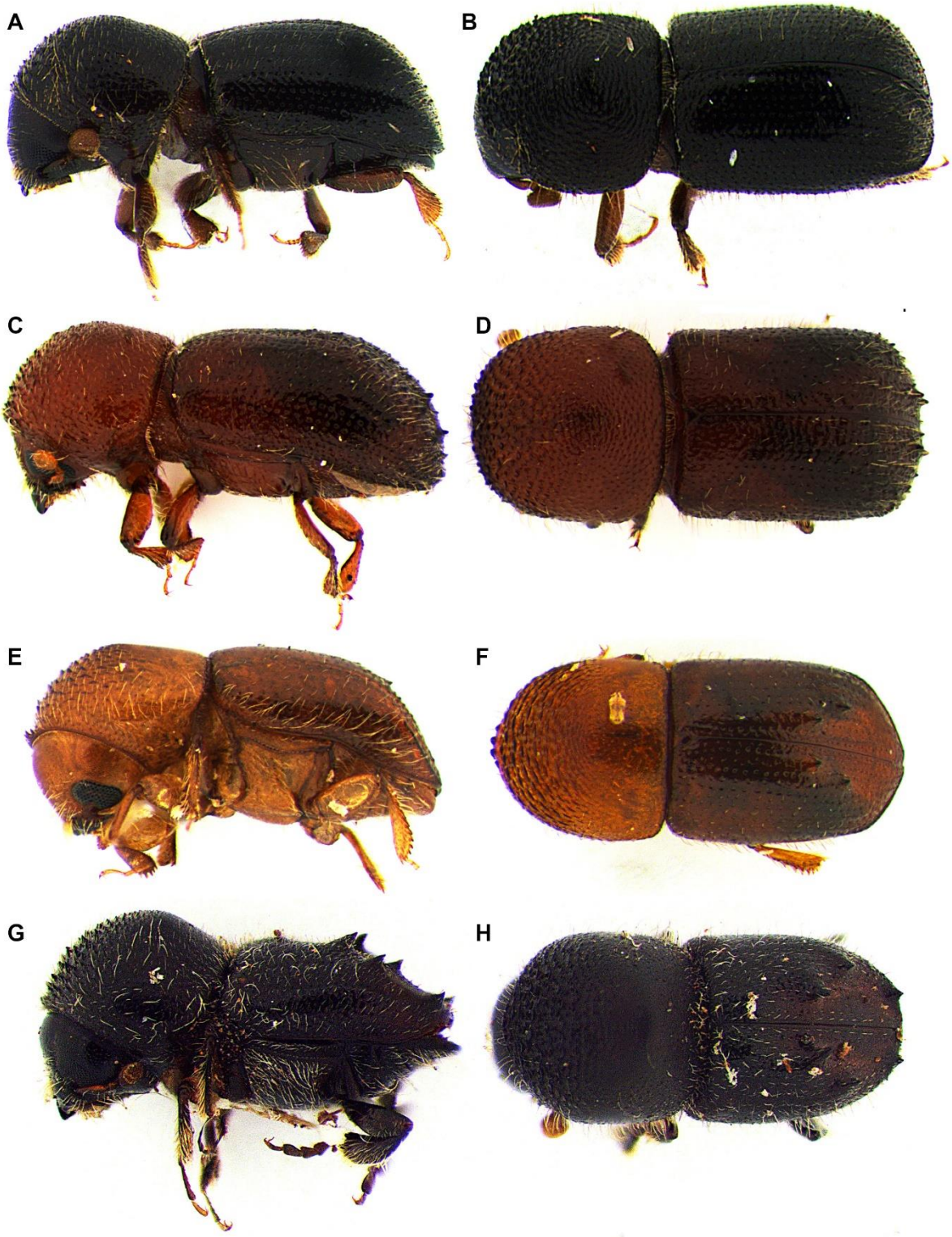

Figure 18. Lateral and dorsal views of Xyleborini species. (A-B) *Ambrosiodmus tachygraphus*, (C-D) *A. pithecolobius*, (E-F) *Diuncus haberkorni*, (G-H) *Eccoptopterus spinosus*.

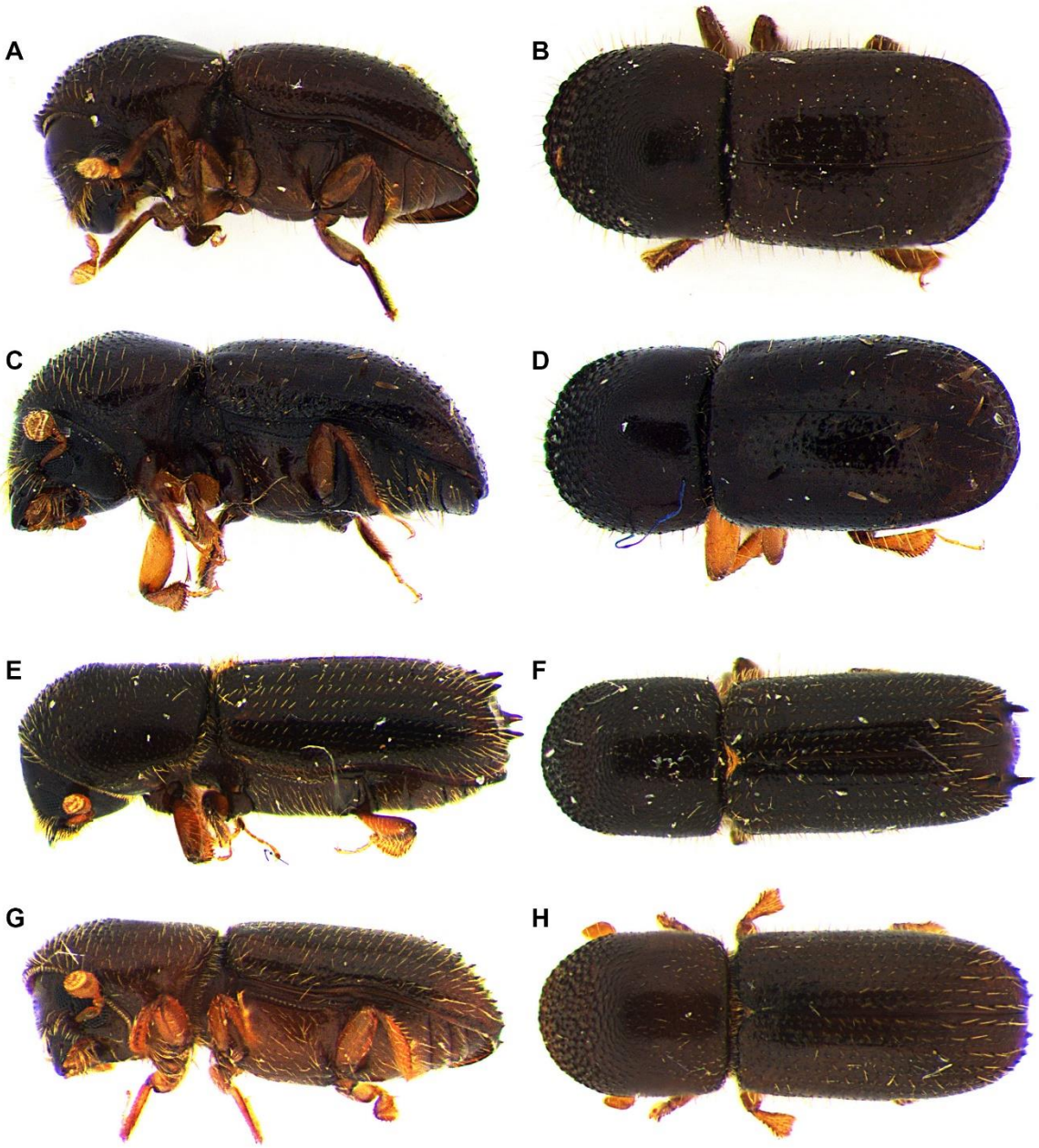

Figure 19. Lateral and dorsal views of Xyleborini species (cont.). (A-B) *Euwallacea fornicatus*, (C-D) *E. xanthopus*, (E-F) *Xyleborinus aemulus.* (G-H) *Xyleborinus saxesenii*,

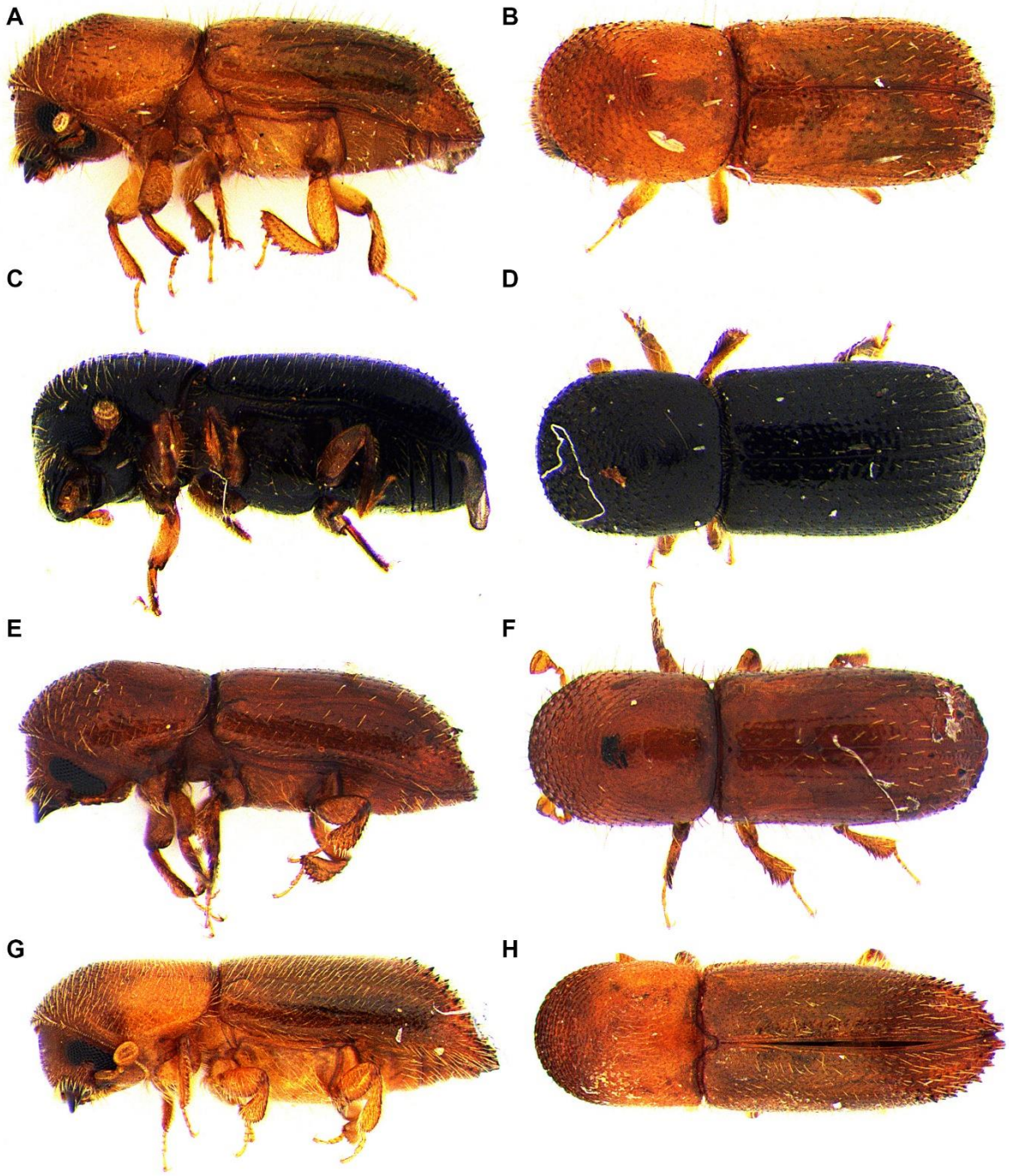

Figure 20. Lateral and dorsal views of Xyleborini (cont.). (A-B) *Xyleborus affinis*, (C-D) *X. antaisaka*, (E-F) *X. ferrugineus*, (G-H) *X. perdiligens*.

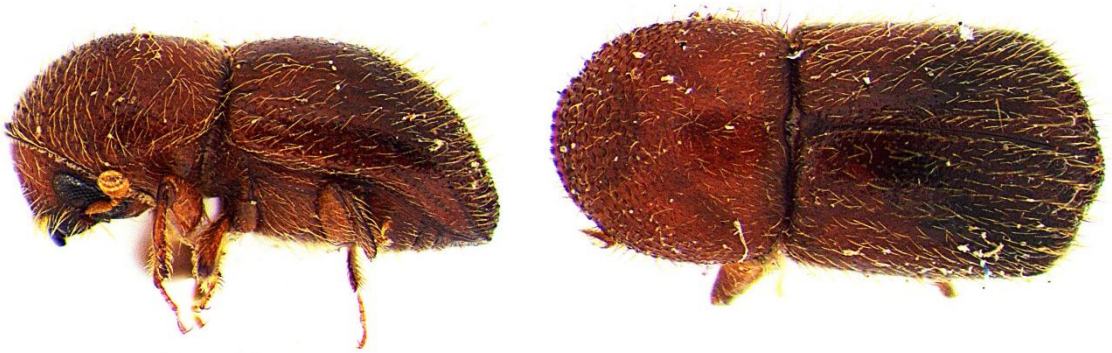

Figure 21. Lateral and dorsal views of *Xylosandrus crassiusculus*.

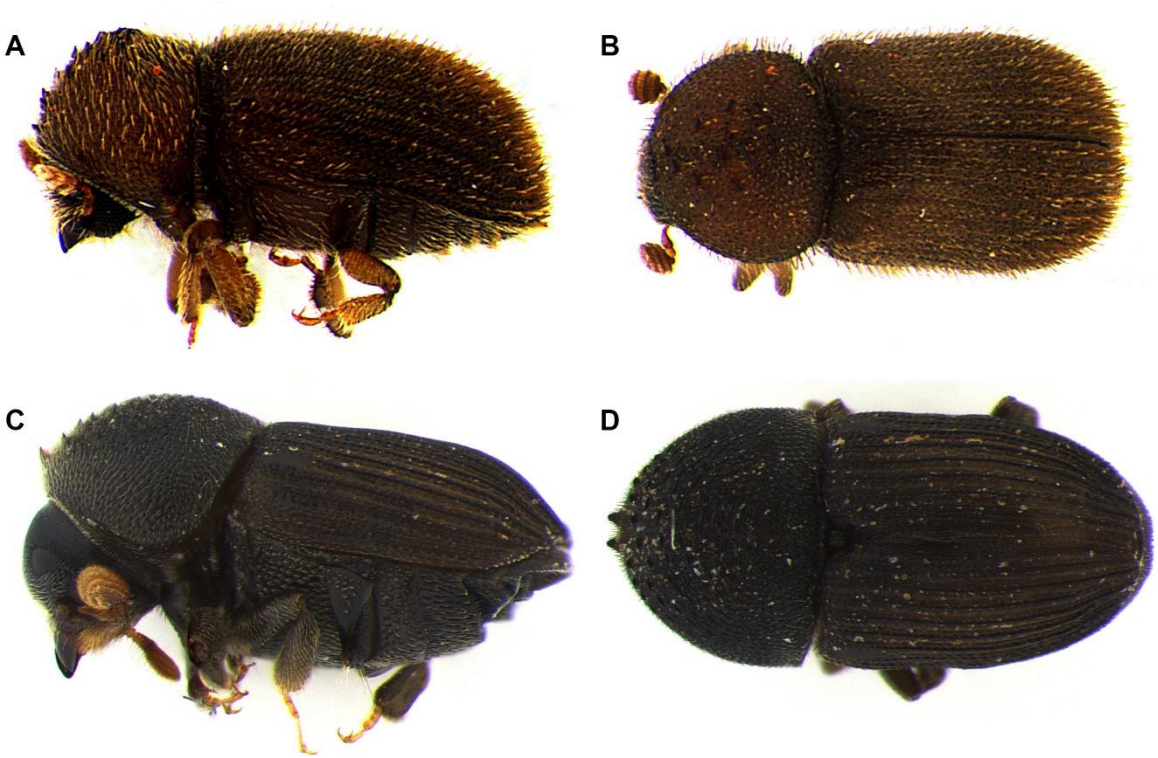

Figure 22. Lateral and dorsal views of Xyloctonini. (A-B) *Glostatus squamosus*. (C-D) *Xyloctonus scolytoides*.

References

Al Adawi, A.O., Al Jabri, R., Deadman, M., Barnes, I., Wingfield, B.D. & Wingfield, M.J. (2013). The mango sudden decline pathogen, *Ceratocystis manginecans*, is vectored by *Hypocryphalus mangiferae* (Coleoptera: Scolytinae) in Oman. *European Journal of Plant Pathology*, 135, 243–251.
DOI: https://doi.org/10.1007/s10658-012-0081-7

Alonso-Zarazaga, M.A. & Lyal, C.H. (2009). A catalogue of family and genus group names in Scolytinae and Platypodinae with nomenclatural remarks (Coleoptera: Curculionidae). *Zootaxa*, 2258, 1–134.
DOI: https://doi.org/10.11646/zootaxa.2258.1.1

Bateman, C., Huang, Y.T., Simmons, D.R., Kasson, M.T., Stanley, E.L. & Hulcr, J. (2017). Ambrosia beetle *Premnobius cavipennis* (Scolytinae: Ipini) carries highly divergent ascomycotan ambrosia fungus, *Afroraffaelea ambrosiae* gen. nov. et sp. nov. (Ophiostomatales). Fungal Ecology, 25, 41-49.
DOI: http://dx.doi.org/10.1016/j.funeco.2016.10.008

Batra, L.R. (1967). Ambrosia fungi: a taxonomic revision, and nutritional studies of some species. *Mycologia*, 59, 976-1017.
DOI: https://doi.org/10.1080/00275514.1967.12018485

Beaver, R.A. (1998). New synonymy, new combinations and taxonomic notes on Scolytidae and Platypodidae (Insecta: Coleoptera). *Annalen des Naturhistorischen Museums in Wien*, 100B, 179–192.

Beaver, R.A. (2010). Taxonomic notes on the afrotropical genera *Hapalogenius* Hagedorn, *Hylesinopsis* Eggers, and *Rhopalopselion* Hagedorn (Coleoptera, Curculionidae, Scolytinae). *ZooKeys*, 56, 157–170.
DOI: https://doi.org/10.3897/zookeys.56.523

Beaver, R.A. (2011). New synonymy and taxonomic changes in bark and ambrosia beetles. *Koleopterologische Rundschau*, 81, 277–289.

Beaver, R.A. & Löyttyniemi, K. (1985). The platypodid ambrosia beetles of Zambia (Coleoptera: Platypodidae). *Revue de Zoologie Africaine*, 99, 113–134.

Belshaw, R. & Quicke, D.L. (1997). A molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae). *Molecular Phylogenetics and Evolution*, 7, 281–293.
DOI: https://doi.org/10.1006/mpev.1996.0400

Bickerstaff, J.R., Smith, S.S., Kent, D.S., Beaver, R.A., Seago, A.E. & Riegler, M. (2020). A review of the distribution and host plant associations of the platypodine ambrosia beetles (Coleoptera: Curculionidae: Platypodinae) of Australia, with an electronic species identification key. *Zootaxa*, 4894, 69-80.
DOI: https://doi.org/10.11646/zootaxa.4894.1.3

Boland, J.M. (2016). The impact of an invasive ambrosia beetle on the riparian habitats of the Tijuana River Valley, California. *PeerJ*, 4, e2141.
DOI: https://doi.org/10.7717/peerj.2141

Brader, L. (1964). Etude de la relation entre le scolyte des rameaux du cafeier, *Xyleborus compactus* Eichh. (*X. morstatti* Hag.), et sa plante-hote. Thesis, Veenman.

Bright, D.E. (2014). A catalog of Scolytidae and Platypodidae (Coleoptera), Supplement 3 (2000-2010), with notes on subfamily and tribal reclassifications. *Insecta Mundi*, *861*.
Link: https://digitalcommons.unl.edu/insectamundi/861/

Bright, D.E. (2021) *Catalog of Scolytidae (Coleoptera), Supplement 4 (2011-2019) With An Annotated Checklist Of The World Fauna (Coleoptera: Curculionoidea: Scolytidae), A*. Colorado State University.
Link: https://hdl.handle.net/10217/229307

Brockerhoff, E.G., Knížek, M. & Bain, J. (2003). Checklist of indigenous and adventive bark and ambrosia beetles (Curculionidae: Scolytinae and Platypodinae) of New Zealand and interceptions of exotic species (1952-2000). *New Zealand Entomologist*, 26, 29-44.
DOI: https://doi.org/10.1080/00779962.2003.9722106

Brockerhoff, E.G., Bain, J., Kimberley, M. & Knížek, M. (2006). Interception frequency of exotic bark and ambrosia beetles (Coleoptera: Scolytinae) and relationship with establishment in New Zealand and worldwide. *Canadian Journal of Forest Research*, 36, 289–298.
DOI: https://doi.org/10.1139/x05–250

Browne, F.G. (1963). Notes on the habits and distribution of some Ghanaian bark beetles and ambrosia beetles (Coleoptera: Scolytidae and Platypodidae). *Bulletin of Entomological Research*, 54, 229–266.
DOI: https://doi.org/10.1017/S0007485300048768

Bush, S.J., Slippers, B., Neser, S., Harney, M., Dittrich-Schröder, G. & Hurley, B.P. (2016). Six recently recorded Australian insects associated with *Eucalyptus* in South Africa. *African Entomology*, 24, 539-544.
DOI: https://doi.org/10.4001/003.024.0539

Colunga–Garcia, M., Haack, R.A. & Adelaja, A.O. (2009). Freight transportation and the potential for invasions of exotic insects in urban and periurban forests of the United States. *Journal of Economic Entomology*, 102, 237–246.
DOI: https://doi.org/10.1603/029.102.0133

Cognato, A.I. (2006). Standard percent DNA sequence difference for insects does not predict species boundaries. *Journal of Economic Entomology*, 99, 1037-1045.
DOI: https://doi.org/10.1093/jee/99.4.1037

Cognato A (2013). Molecular phylogeny and taxonomic review of Premnobiini Browne, 1962 (Coleoptera: Curculionidae: Scolytinae). *Frontiers in Ecology and Evolution*, 1, 1-12.
DOI: https://doi.org/10.3389/fevo.2013.00001

Cognato, A.I., Sari, G., Smith, S.M., Beaver, R.A., Li, Y., et al. (2020). The essential role of taxonomic expertise in the creation of DNA databases for the identification and delimitation of Southeast Asian ambrosia beetle species (Curculionidae: Scolytinae: Xyleborini). *Frontiers in Ecology and Evolution*, 8.
DOI: https://doi.org/10.3389/fevo.2020.00027

Cruz, L.F., Menocal, O., Mantilla, J., Ibarra-Juarez, L.A. & Carrillo, D. (2019). *Xyleborus volvulus* (Coleoptera: Curculionidae): biology and fungal associates. *Applied and Environmental Microbiology*, 85, e01190-01119.
DOI: https://doi.org/ 10.1128/AEM.01190-19

Davydenko, K., Vasaitis, R., Meshkova, V. & Menkis, A. (2014). Fungi associated with the red-haired bark beetle, *Hylurgus ligniperda* (Coleoptera: Curculionidae) in the forest-steppe zone in eastern Ukraine. *European Journal of Entomology*, 111, 561-565.
DOI: https://doi.org/ 10.14411/eje.2014.070

De Errasti, A., De Beer, Z.W., Coetzee, M.P., Roux, J., Rajchenberg, M. & Wingfield, M.J. (2016). Three new species of Ophiostomatales from *Nothofagus* in Patagonia. *Mycological Progress*, 15, 17.
DOI: https://doi.org/10.1007/s11557-016-1158-z

Dole, S.A. & Beaver, R.A. (2008). A review of the Australian species of *Xylosandrus* Reitter (Coleoptera: Curculionidae: Scolytinae). *The Coleopterists Bulletin*, 62, 481-492.
DOI: https://doi.org/10.1649/1108.1

Dole, S.A. & Cognato, A.I. (2010). Phylogenetic revision of *Xylosandrus* Reitter (Coleoptera: Curculionidae: Scolytinae: Xyleborina). *Proceedings of the California Academy of Sciences*, 61, 451.

Doganlar, M. & Schopf, R. (1984). Some biological aspects of the European oak bark beetle, *Scolytus intricatus* (Ratz.) (Col., Scolytidae) in the northern parts of Germany (BRD). *Zeitschrift für Angewandte Entomologie*, 97: 153-162.
DOI: https://doi.org/10.1111/j.1439-0418.1984.tb03731.x

Faccoli, M. (2008). First record of *Xyleborus atratus* Eichhoff from Europe, with an illustrated key to the European Xyleborini (Coleoptera: Curculionidae: Scolytinae). *Zootaxa*, 1772, 55–62.
DOI: https://doi.org/10.11646/zootaxa.1772.1.2

Faccoli, M., Gallego, D., Branco, M., Brockerhoff, E.G., Corley, J., et al. (2020). A first worldwide multispecies survey of invasive Mediterranean pine bark beetles (Coleoptera: Curculionidae, Scolytinae). *Biological Invasions*, 22, 1785-799.
DOI: https://doi.org/10.1007/s10530-020-02219-3

Freeman, S., Protasov, A., Sharon, M., Mohotti, K., Eliyahu, M., et al. (2012). Obligate feed requirement of *Fusarium* sp. nov., an avocado wilting agent, by the ambrosia beetle *Euwallacea aff. fornicata*. *Symbiosis*, 58, 245-251.
DOI: https://doi.org/10.1007/s13199-013-0222-6

Galdino, T.V.S., Kumar, S., Oliveira, L.S.S., Alfenas, A.C., Neven, L.G., et al. (2016) Mapping global potential risk of Mango Sudden Decline Disease caused by *Ceratocystis fimbriata*. *PLoS ONE*, 11, e0159450.
DOI: https://doi.org/10.1371/journal.pone.0159450

Gomez, D.F., Rabaglia, R.J., Fairbanks, K. & Hulcr, J. (2018). North American Xyleborini north of Mexico: a review and key to genera and species (Coleoptera, Curculionidae, Scolytinae). *ZooKeys*, 768, 19–68.
DOI:https://doi.org/10.3897/zookeys.768.24697

Gomez, D.F., Johnson, A.J., De Grammont, P.C., Alfonso-Simonetti, J., Montaigne, J. et al. (2020). New records of bark and ambrosia beetles (Coleoptera: Scolytinae) from Cuba with description of a new species. *Florida Entomologist*, 102, 717-724.
DOI: https://doi.org/10.1653/024.102.0408.

Grégoire, J–C., Raffa, K.F. & Lindgren, B.S. (2015). Chapter 15 – Economics and Politics of Bark Beetles. In: Vega, F.E. & Hofstetter, R.W. (Eds), *Bark Beetles.* Academic Press, San Diego, pp. 585–613.
DOI: https://doi.org/10.1016/B978-0-12-417156-5.00015-0

Grousset, F., Grégoire, J.C., Jactel, H., Battisti, A., Benko Beloglavec, A. et al. (2020). The risk of bark and ambrosia beetles associated with imported non-coniferous wood and potential horizontal phytosanitary measures. *Forests*, 11, 342.
DOI: https://doi.org/10.3390/f11030342

Haack, R.A. (2006). Exotic bark–and wood–boring Coleoptera in the United States: recent establishments and interceptions. *Canadian Journal of Forest Research*, 36, 269–288.
DOI: https://doi.org/10.1139/x05-249

Harrington, T.C., Aghayeva, D. & Fraedrich, S.W. (2010). New combinations in *Raffaelea*, *Ambrosiella*, and *Hyalorhinocladiella*, and four new species from the redbay ambrosia beetle, *Xyleborus glabratus*. *Mycotaxon*, 111, 337-361.
DOI: https://doi.org/10.5248/111.337

Harrington, T.C., McNew, D., Mayers, C., Fraedrich, S.W. & Reed, S.E. (2014). *Ambrosiella roeperi* sp. nov. is the mycangial symbiont of the granulate ambrosia beetle, *Xylosandrus crassiusculus*. *Mycologia*, 106, 835-845.
DOI: https://doi.org/10.3852/13-354

Hulcr, J. & Cognato, A.I. (2009). Three new genera of oriental Xyleborina (Coleoptera: Curculionidae: Scolytinae). *Zootaxa*, 2204, 19–36.
DOI: https://doi.org/10.11646/zootaxa.2204.1.2

Hulcr, J. & Cognato, A.I. (2010). Repeated evolution of crop theft in fungus-farming ambrosia beetles. *Evolution: International Journal of Organic Evolution*, 64, 3205–3212.
DOI: https://doi.org/10.1111/j.1558–5646.2010.01055.x

Hulcr, J. & Stelinski, L.L. (2017). The ambrosia symbiosis: from evolutionary ecology to practical management. *Annual Review of Entomology*, 62, 285–303.
DOI: https://doi.org/10.1146/annurev-ento-031616-035105

Hulcr, J., Dole, S.A., Beaver, R.A. & Cognato, A.I. (2007). Cladistic review of generic taxonomic characters in Xyleborina (Coleoptera: Curculionidae: Scolytinae). *Systematic Entomology*, 32, 568–584.
DOI: https://doi.org/10.1111/j.1365-3113.2007.00386.x

Hulcr, J., Atkinson, T.H., Cognato, A.I., Jordal, B.H. & McKenna, D.D. (2015). Chapter 2 – Morphology, taxonomy, and phylogenetics of bark beetles. In: Vega, F.E. & Hofstetter, R.W. (Eds), *Bark Beetles.* Academic Press, San Diego, pp. 41–84.
DOI: https://doi.org/10.1016/B978-0-12-417156-5.00002-2

Hulcr, J., Black, A., Prior, K., Chen, C-Y. & Li, H-F. (2017). Studies of ambrosia beetles (Coleoptera: Curculionidae) in their native ranges help predict invasion impact. *Florida Entomologist*, 100, 257–261.
DOI: https://doi.org/10.1653/024.100.0219

Hurley, B.P., Garnas, J., Wingfield, M.J., Branco, M., Richardson, D.M. & Slippers, B. (2016). Increasing numbers and intercontinental spread of invasive insects on eucalypts. *Biological invasions*, 18, 921-933.
DOI: https://doi.org/10.1007/s10530-016-1081-x

Hurley, B.P., Slippers, B., Sathyapala, S. & Wingfield, M.J. (2017). Challenges to planted forest health in developing economies. Biological invasions, 19, 3273-3285.
DOI: https://doi.org/10.1007/s10530-017-1488-z

Jactel, H., Desprez-Loustau, M.L., Battisti, A., Brockerhoff, E., Santini, A., et al. (2020). Pathologists and entomologists must join forces against forest pest and pathogen invasions. *NeoBiota*, 58, 107-127.
DOI: https://doi.org/10.3897/neobiota.58.54389

Jankowiak, R. (2006). Mycobiota associated with *Hylurgops palliatus* [Gyll.] on *Pinus sylvestris* L. in Poland. *Acta Societatis Botanicorum Poloniae*, 75, 333-338.
DOI: https://doi.org/10.5586/asbp.2006.040

Jankowiak, R., & Bilański, P. (2013). Ophiostomatoid fungi associated with root-feeding bark beetles on Scots pine in Poland. *Forest Pathology*, 43, 422-428.
DOI: https://doi.org/10.1111/efp.12049

Johnson, A.J., Hulcr, J., Knížek, M., Atkinson, T.H., Mandelshtam, M.Y., et al. (2020). Revision of the bark beetle genera within the former Cryphalini (Curculionidae: Scolytinae). *Insect Systematics and Diversity*, 4, 1.
DOI: https://doi.org/10.1093/isd/ixaa002

Jordal, B. H. (2009). Two new species of *Aphanarthrum* Wollaston (Coleoptera: Curculionidae, Scolytinae) associated with *Euphorbia* in South Africa. *Zootaxa*, 1983, 54-62.

DOI: https://doi.org/10.11646/zootaxa.1983.1.4

Jordal, B.H. & Kambestad, M. (2014). DNA barcoding of bark and ambrosia beetles reveals excessive NUMTs and consistent east-west divergence across Palearctic forests. *Molecular Ecology Resources*, 14, 7-17.
DOI: https://doi.org/10.1111/1755-0998.12150

Kanzi, A.M., Trollip, C., Wingfield, M.J., Barnes, I., Van Der Nest, M.A. & Wingfield, B.D. (2020). Phylogenomic incongruence in *Ceratocystis*: a clue to speciation? *BMC Genomics*, 21, 362.
DOI: https://doi.org/10.1186/s12864-020-6772-0

Kim, S., Harrington, T.C., Lee, J.C. & Seybold, S.J. (2011). *Leptographium tereforme* sp. nov. and other Ophiostomatales isolated from the root-feeding bark beetle *Hylurgus ligniperda* in California. *Mycologia*, 103, 152-163.
DOI: https://doi.org/10.3852/10-096

Krokene, P. & Solheim, H. (1998). Pathogenicity of four blue-stain fungi associated with aggressive and nonaggressive bark beetles. *Phytopatholog*, 88, 39-44.
doi:10.1094/phyto.1998.88.1.39

Landi, L., Gómez, D., Braccini, C.L., Pereyra, V.A., Smith, S.M. & Marvaldi, A.E. (2017). Morphological and molecular identification of the invasive *Xylosandrus crassiusculus* (Coleoptera: Curculionidae: Scolytinae) and its South American range extending into Argentina and Uruguay. *Annals of the Entomological Society of America*, 110, 344–349.
DOI: https://doi.org/10.1093/aesa/sax032

Liebhold, A.M., Brockerhoff, E.G., Garrett, L.J., Parke, J.L. & Britton, K.O. (2012). Live plant imports: the major pathway for forest insect and pathogen invasions of the US. *Frontiers in Ecology and the Environment*, 10, 135–143.
DOI: https://doi.org/10.1890/110198

Lin, Y.T., Shih, H.H., Hulcr, J., Lin, C.S., Lu, S.S. & Chen, C.Y. (2017). *Ambrosiella* in Taiwan including one new species. *Mycoscience*, 58, 242-252.
DOI: https://doi.org/10.1016/j.myc.2017.02.004

Lukić I, Zgrablić Ž & Mičetić Stanković V (2019). Presence of birch bark beetle (*Scolytus ratzeburgi*) in Croatia. Šumarski list 143: 523-528.
DOI: https://doi.org/10.31298/sl.143.11-12.2

Lynch, S.C., Twizeyimana, M., Mayorquin, J.S., Wang, D. H., Na, F., et al. (2016). Identification, pathogenicity and abundance of *Paracremonium pembeum* sp. nov. and *Graphium euwallaceae* sp. nov.—two newly discovered mycangial associates of the polyphagous shot hole borer (*Euwallacea* sp.) in California. *Mycologia*, 108, 313-329.
DOI: https://doi.org/10.3852/15-063

Mandelshtam, M.Y. & Beaver, R.A. (2003). *Amphiscolytus*–a new genus, and Amphiscolytini–a new tribe of Scolytidae (Coleoptera) for *Dacryophthorus capensis* (Schedl). *Zootaxa*, 298, 1–8.
DOI: https://doi.org/10.11646/zootaxa.298.1.1

Mandelshtam, M.Y. & Danielsson, R. (2004). Some African Scolytidae (Coleoptera) collected by Swedish entomological expeditions. *Annals of the Transvaal Museum*, 41, 90–92.

Mandelshtam, M.Y. & Selikhovkin, A. (2020). Bark and ambrosia beetles (Coleoptera, Curculionidae: Scolytinae) of Northwest Russia: History of the study, composition and genesis of the fauna. *Entomological Review*, 100, 800-826.
DOI: https://doi.org/10.1134/S0013873820060093

Maniatis, T., Fritsch, E.F. & Sambrook, J. (1983) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbour Laboratory, Cold Spring Harbour, New York.

Martínez, M., Cognato, A.I., Guachambala, M. & Boivin, T. (2019). Bark and ambrosia beetle (Coleoptera: Curculionidae: Scolytinae) diversity in natural and plantation forests in Ecuador. Environmental Entomology, 48, 603-613.
DOI: https://doi.org/10.1093/ee/nvz037

Masood, A. & Saeed, S. (2012). Bark beetle, *Hypocryphalus mangiferae* Stebbing (Coleoptera: Curculionidae: Scolytinae) is a vector of mango sudden death disease in Pakistan. *Pakistan Journal of Botany*, 44, 813–820.

Mayers, C., Harrington, T.C., Masuya, H., Jordal, B., McNew, D., et al. (2020). Patterns of coevolution between ambrosia beetle mycangia and the Ceratocystidaceae, with five new fungal genera and seven new species. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, 44, 41-66.
DOI: https://doi.org/10.3767/persoonia.2020.44.02

Meiklejohn, K.A., Damaso, N. & Robertson, J.M. (2019). Assessment of BOLD and GenBank – Their accuracy and reliability for the identification of biological materials. *PLoS ONE*, 14, e0217084.
DOI: https://doi.org/10.1371/journal.pone.0217084

Mendel, Z. (1984). Life history of *Phloeosinus armatus* Reiter and *P. aubei* Perris (Coleoptera: Scolytidae) in Israel. *Phytoparasitica*, 12, 89–97.

Meyerson, L.A. & Mooney, H.A. (2007). Invasive alien species in an era of globalization. *Frontiers in Ecology and the Environment*, 5, 199–208.
DOI: https://doi.org/10.1890/1540-9295(2007)5[199:IASIAE]2.0.CO,2

Meurisse, N., Rassati, D., Hurley, B.P., Brockerhoff, E.G. & Haack, R.A. (2019). Common pathways by which non-native forest insects move internationally and domestically. *Journal of Pest Science*, 92, 13–27.
https://doi.org/10.1007/s10340-018-0990-0

Mitchell, A. & Maddox, C. (2010). Bark beetles (Coleoptera: Curculionidae: Scolytinae) of importance to the Australian macadamia industry: an integrative taxonomic approach to species diagnostics. *Australian Journal of Entomology*, 49, 104-113.
DOI: https://doi.org/10.1111/j.1440-6055.2010.00746.x

Musvuugwa, T., De Beer, Z.W., Duong, T.A., Dreyer, L.L., Oberlander, K.C. & Roets, F. (2015). New species of Ophiostomatales from Scolytinae and Platypodinae beetles in the Cape Floristic Region, including the discovery of the sexual state of *Raffaelea*. *Antonie van Leeuwenhoek*, 108, 933-950.
DOI: https://doi.org/10.1007/s10482-015-0547-7

Nel W.J., Wingfield M.J., De Beer Z.W. & Duong T.A. (2020). The granulate ambrosia beetle, *Xylosandrus crassiusculus* (Coleoptera: Curculionidae, Scolytinae), and its fungal symbiont found in South Africa. *Zootaxa* 4838, 427-435.
DOI: https://doi.org/10.11646/zootaxa.4838.3.7

Nel, W.J., Wingfield, M.J., De Beer, Z.W., & Duong, T.A. (2021). Ophiostomatalean fungi associated with wood boring beetles in South Africa including two new species. *Antonie van Leeuwenhoek*. [Online]
DOI: 10.1007/s10482-021-01548-0

Nilsson, R.H., Ryberg, M., Kristiansson, E., Abarenkov, K., Larsson, K.H. & Kõljalg, U. (2006). Taxonomic reliability of DNA sequences in public sequence databases: A fungal perspective. *PLoS ONE*, 1, e59.
DOI: https://doi.org/10.1371/journal.pone.0000059

Ohsawa, M., Langor, D., Hiratsuka, Y. & Yamaoka, Y. (2000). Fungi associated with *Dendroctonus rufipennis* and *Polygraphus rufipennis*, and white spruce inoculation tests. *Canadian Journal of Plant Pathology*, 22, 254-257.
DOI: https://doi.org/ 10.1080/07060660009500472

Olson, D.M., Dinerstein, E., Wikramanayake, E.D., Burgess, N.D., Powell et al. (2001). Terrestrial ecoregions of the world: A new map of life on earth: A new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. *BioScience*, 51, 933-938. DOI: https://doi.org/10.1641/0006-3568(2001)051[0933:Teotwa]2.0.Co,2

Oliveira, L.S.S, Harrington, T.C., Ferreira, M.A., Damacena, M.B., Al-Sadi, A.M., et al. (2015). Species or genotypes? Reassesment of four recently described species of the *Ceratocystis* wilt pathogen, *Ceratocystis fimbriata*, on *Mangifera indica*. *Phytopathology*, 105, 1229-1244. DOI: https://doi.org/10.1094/PHYTO-03-15-0065-R

Paap, T., De Beer Z.W., Migliorini, D., Nel, W.J. & Wingfield, M.J. (2018). The polyphagous shot hole borer (PSHB) and its fungal symbiont *Fusarium euwallaceae*: a new invasion in South Africa. *Australasian Plant Pathology*, 47, 231–237. DOI: https://doi.org/10.1007/s13313-018-0545-0

Paap, T., Wingfield, M.J., Burgess, T.I., Hulbert, J.M. & Santini A. (2020). Harmonising the field of invasion science and forest pathology. *NeoBiota*, 62, 301-332. DOI: https://doi.org/10.3897/neobiota.62.52991

Paini, D.R., Sheppard, A.W., Cook, D.C., De Barro, P.J., Worner, S.P. & Thomas, M.B. (2016). Global threat to agriculture from invasive species. *Proceedings of the National Academy of Sciences*, 113, 7575-7579. DOI: https://doi.org/10.1073/pnas.1602205113

Pennacchio, F., Danti, R., Benassai, D., Squarcini, M., Marziali, L., et al. (2013). A new additional record of *Phloeosinus armatus* Reitter from Italy (Coleoptera Curculionidae Scolytinae). *Redia*, 96, 45–50.

Persson, Y., Vasaitis, R., Långström, B., Öhrn, P., Ihrmark, K. & Stenlid, J. (2009). Fungi vectored by the bark beetle *Ips typographus* following hibernation under the bark of standing trees and in the forest litter. *Microbial Ecology*, 58, 651-659. DOI: https://doi.org/10.1007/s00248-009-9520-1

Ploetz, R.C., Hulcr, J., Wingfield, M.J. & De Beer, Z.W. (2013). Destructive tree diseases associated with ambrosia and bark beetles: black swan events in tree pathology? *Plant Disease*, 97, 856–872. DOI: https://doi.org/10.1094/PDIS-01-13-0056-FE

Pyšek, P., Hulme, P.E., Simberloff, D., Bacher, S., Blackburn, T.M., et al. (2020) Scientists' warning on invasive alien species. *Biological Reviews*, 95, 1511-1534. DOI: https://doi.org/ 10.1111/brv.12627

Rabaglia, R.J., Dole, S.A. & Cognato, A.I. (2006). Review of American Xyleborina (Coleoptera: Curculionidae: Scolytinae) occurring north of Mexico, with an illustrated key. *Annals of the Entomological Society of America*, 99, 1034–1056. DOI: https://doi.org/10.1603/0013-8746(2006)99[1034:ROAXCC]2.0.CO,2

Rabaglia, R.J., Beaver, R.A., Johnson, A.J., Schmaedick, M.A. & Smith, S.M. (2020). The bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae and Platypodinae) of American Samoa. *Zootaxa*, 4808, 171-195. DOI: https://doi.org/10.11646/zootaxa.4808.1.11

Raja, H. A., Miller, A.N., Pearce, C.J. & Oberlies, N.H. (2017). Fungal identification using molecular tools: A primer for the natural products research community. *Journal of Natural Products*, 80, 756-770. DOI: https://doi.org/10.1021/acs.jnatprod.6b01085

Reay, S., Thwaites, J. & Farrell, R. (2005). A survey of Ophiostoma species vectored by *Hylastes ater* to pine seedlings in New Zealand. *Forest Pathology*, 35, 105-113. DOI: http://dx.doi.org/10.1111/j.1439-0329.2004.00393.x

Romon, P., Zhou, X., Iturrondobeitia, J.C., Wingfield, M.J. & Goldarazena, A. (2007). *Ophiostoma* species (Ascomycetes: Ophiostomatales) associated with bark beetles (Coleoptera: Scolytinae) colonizing *Pinus radiata* in northern Spain. *Canadian Journal of Microbiology*, 53, 756-767. DOI: https://doi.org/10.1139/W07-001

Roux, J., Hurley, B.P., Wingfield, M.J., Bredenkamp, B. & Upfold, S. (2012). Diseases and pests of eucalypts, pines and wattle. *South African Forestry Handbook*, 303-335.

SAMGA (2020). Southern African Mango Growers' Association. Available from: https://www.mango.co.za/about-us/ [Accessed 31 July 2020]

Saucedo-Carabez, J., Ploetz, R.C., Konkol, J., Carrillo, D. & Gazis, R. (2018). Partnerships between ambrosia beetles and fungi: lineage-specific promiscuity among vectors of the laurel wilt pathogen, *Raffaelea lauricola*. *Microbial Ecology*, 76, 925-940. DOI: https://doi.org/10.1007/s00248-018-1188-y

Sandoval Rodríguez, C., Cognato, A.I. & Righi, C.A. (2017). Bark and ambrosia beetle (Curculionidae: Scolytinae) diversity found in agricultural and fragmented forests in Piracicaba-SP, Brazil. *Environmental Entomology*, 46, 1254-1263. DOI: https://doi.org/10.1093/ee/nvx160

Schedl KE (1955) XXVIII.—Contributions to the morphology and taxonomy of the Scolytoidea: 147. New records and new species of Scolytoidea from Africa. *Journal of Natural History*, 8, 211-220.

Schedl, K.E. (1957). Bark– and timber–beetles from South Africa. *Annals and Magazine of Natural History*, 10, 149–159. DOI: https://doi.org/10.1080/00222935708655940

Schedl, K.E. (1960). On two new collections of African Scolytidae: 187. Contribution to the Morphology and Taxonomy of the Scolytoidea. *Journal of Natural History*, 3, 349–352.

Schedl, K.E. (1965) South African bark and timber beetles. *Journal of the Entomological Society of Southern Africa*, 28, 110–116.

Schedl, K.E. (1970). South African bark and timber beetles. *Annals of the Transvaal Museum*, 26, 177–182.

Schedl, K.E. (1975). South African bark and timber beetles, 3. Contribution to the morphology and taxonomy of the Scolytoidea. *Annals of the Transvaal Museum*, 29, 275–281.

Schedl, K.E. (1982). Scolytoidea (Coleoptera), mainly from South Africa. *Annals of the Transvaal Museum*, 33, 277–286.

Scott, D.B. & Du Toit, J. (1970). Three new *Raffaelea* species. *Transactions of the British Mycological Society*, 55, 181-186. DOI: https://doi.org/10.1016/S0007-1536(70)80002-X

Seifert, K.A. (1993). Sapstain of commercial lumber by species of *Ophiostoma* and *Ceratocystis*. In: Wingfield, M.J, Seifert, K.A., & Webber, J. (Eds.), *Ceratocystis and Ophiostoma: Taxonomy, Ecology and Pathogenicity*. APS Press, St. Paul, Minnesota, pp. 141–151.

Shimizu, A., Tanaka, R., Akiba, M., Masuya, H., Iwata, R., et al. (2013). Nematodes associated with *Dryocoetes uniseriatus* (Coleoptera: Scolytidae). *Environmental entomology*, 42, 79–88. DOI: https://doi.org/10.1603/EN12165

Six, D., De Beer, Z.W., Beaver, R.A., Visser, L. & Wingfield, M.J. (2005). Exotic invasive elm bark beetle, *Scolytus kirschii*, detected in South Africa. *South African Journal of Science*, 101, 229-232.

Smith, S.M. & Cognato, A.I. (2014). A taxonomic monograph of Nearctic *Scolytus* Geoffroy (Coleoptera, Curculionidae, Scolytinae). ZooKeys 1, 1-182. DOI: https://doi.org/10.3897/zookeys.450.7452

Susaeta, A., Soto, J.R., Adams, D.C. & Hulcr, J. (2016). Pre-invasion economic assessment of invasive species prevention: A putative ambrosia beetle in Southeastern loblolly pine forests. *Journal of Environmental Management*, 183, 875-881.
DOI: https://doi.org/10.1016/j.jenvman.2016.09.037

Van Der Linde, J.A., Six, D.L., Wingfield, M.J. & Roux, J. (2018). Fungi and insects associated with *Euphorbia ingens* die–off in South Africa. *Southern Forests: A Journal of Forest Science*, 80, 21–28.
DOI: https://doi.org/10.2989/20702620.2016.1263004

Van Der Linde, J.A., Six, D.L., De Beer, Z.W., Wingfield, M.J. & Roux, J. (2016). Novel ophiostomatalean fungi from galleries of *Cyrtogenius africus* (Scolytinae) infesting dying *Euphorbia ingens*. *Antonie van Leeuwenhoek*, 109, 589–601.
DOI: https://doi.org/10.1007/s10482-016-0661-1

Van Wyk, M., Al Adawi, A.O., Khan, I.A., Deadman, M.L., Al Jahwari, A.A., et al. (2007). *Ceratocystis manginecans* sp. nov., causal agent of a destructive mango wilt disease in Oman and Pakistan. *Fungal Diversity*, 27, 213–230.

Van Wyk, M., Wingfield, B.D., Al Adawi, A.O., Rossetto, C.J., Ito, M.F. & Wingfield, M.J. (2011). Two new *Ceratocystis* species associated with mango disease in Brazil. *Mycotaxon*, 117, 381–404.
DOI: https://doi.org/10.5248/117.381

Vega, F.E., Infante, F. & Johnson, A.J. (2015.) Chapter 11 – The genus *Hypothenemus*, with emphasis on *H. hampei*, the coffee berry borer. In: Vega, F.E. & Hofstetter, R.W. (Eds.), *Bark Beetles*. Academic Press, San Diego, pp. 427–494.

Weber, B. & McPherson, J. (1983). World list of host plants of *Xylosandrus germanus* (Blandford) (Coleoptera: Scolytidae). *The Coleopterists' Bulletin*, 37, 114–134.

Webber, J. (2004). Experimental studies on factors influencing the transmission of Dutch elm disease. *Forest Systems* 13, 197-205.

Wingfield, M.J., Brockerhoff, E., Wingfield, B.D. & Slippers, B. (2015). Planted forest health: the need for a global strategy. *Science*, 349, 832–836.
DOI: https://doi.org/ 10.1126/science.aac6674

Wingfield, M.J., Slippers, B., Hurley, B.P., Coutinho, T., Wingfield, B.D. & Roux, J. (2008). Eucalypt pests and diseases: growing threats to plantation productivity. *Southern Forests: A Journal of Forest Science*, 70, 139-144.
DOI: https://doi.org/10.2989/SOUTH.FOR.2008.70.2.9.537

Wingfield, M.J., Hurley, B.P. Wingfield, B.D. & Slippers, B. (2020). Tree health in South Africa: Retrospect and prospect. South African Journal of Science, 116. [online]
DOI: https://doi.org/10.17159/sajs.2020/8038

Wood, S.L. (1993). Revision of the genera of Platypodidae (Coleoptera). *The Great Basin Naturalist*, 53, 259–281.

Wood, S.L. (2007). *Bark and ambrosia beetles of South America*. Bean Life Science Museum, Brigham Young University Provo, Utah.

Wood, S.L. & Bright, D.E. (1992). A Catalog of Scolytidae and Platypodidae (Coleoptera), Part 2: Taxonomic Index. *The Great Basin Naturalist Memoirs*, 13, pp. 1–1553.

Yamaoka, Y., Wingfield, M.J., Takahashi, I. & Solheim, H. (1997). Ophiostomatoid fungi associated with the spruce bark beetle *Ips typographus f. aponicus* in Japan. *Mycological research*, 101, 1215-1227.
DOI: https://doi.org/10.1017/S0953756297003924

Zhao, T., Kandasamy, D., Krokene, P., Chen, J., Gershenzon, J. & Hammerbacher, A. (2019). Fungal associates of the tree-killing bark beetle, *Ips typographus*, vary in virulence, ability to degrade

conifer phenolics and influence bark beetle tunneling behavior. *Fungal Ecology*, 38, 71-79. DOI: https://doi.org/10.1016/j.funeco.2018.06.003

Zhou, X.D., De Beer, Z.W., Wingfield, B.D. & Wingfield, M.J. (2001). Ophiostomatoid fungi associated with three pine-infesting bark beetles in South Africa. *Sydowia*, 53, 290-300.

Zwolinski, J. & Geldenhuys, C. (1988). Insect damage to timbers from the indigenous forests—preliminary results. *South African Forestry Journal*, 146, 7–11. DOI: https://doi.org/10.1080/00382167.1988.9630351

SUMMARY

The recent introduction of the devastating Polyphagous Shot Hole Borer (*Euwallacea fornicatus*) and its fungal symbiont (*Fusarium euwallaceae*) into South Africa has led to renewed interest in ambrosia beetles and their symbiotic fungi in the country. However, a quick literature search for studies focussed on these insects and their associated fungi originating from South Africa revealed a large gap in original research. In this study I set out to address some of this knowledge gap, investigating both the ambrosia beetles and their fungal associates. Results from this study shows that South Africa is home to a rich biodiversity of ambrosia beetle species, their closely related bark beetle cousins, and the fungi they associate with.

In recent years, South Africa has become something of a diversity hotspot for Ophiostomatalean fungi, with many species, from a variety of unique niches, having been discovered and described here. Like these previous studies, this study also resulted in the description of five additional new Ophiostomatalean species from the country. Not only that, but three of these species residing in two new genera, were identified from fungus farming termite mounds, a unique and unexplored niche in which to find Ophiostomatalean fungi.

Recent advanced in genomics has made it possible to elucidate the genomic patterns associated with the evolution of various lifestyles. By analysing genome sequence data from many species, I was able to show that a symbiosis with arthropods evolved by various members of the Sordariomycetes, including those with ambrosia beetles, have resulted in losses of genes belonging to many important gene families. Additionally, this study showed that the longer a species has been in symbiosis with its insect partner and the more strictly they associate, oftentimes the more extreme the changes to their genomes are. The initial investigation of this intriguing biological system as part of this PhD serves as an important platform for continuing investigations into this interesting but unexplored relationship.