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ORIGINAL PAPER

The polyphagous shot hole borer (PSHB) and its fungal symbiont *Fusarium euwallaceae*: a new invasion in South Africa

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Abstract
The polyphagous shot hole borer (PSHB), an ambrosia beetle (Coleoptera: Curculeonidae: Scolytinae) native to Asia, together with its fungal symbiont *Fusarium euwallaceae,* has emerged as an important invasive pest killing avocado and other trees in Israel and the United States. The PSHB is one of three cryptic species in the *Euwallacea fornicatus* species complex, the taxonomy of which remains to be resolved. The surge in the global spread of invasive forest pests such as the PSHB has led to the development of programmes utilising sentinel tree plantings to record new host-pest interactions. During routine surveys of tree health in botanical gardens of South Africa undertaken as part of a sentinel project, an ambrosia beetle/fungal associate was detected damaging *Platanus* x *acerifolia* (London Plane) in the KwaZulu-Natal National Botanical Gardens, Pietermaritzburg. Identification of the beetle by sequencing part of the mitochondrial cytochrome oxidase *c* subunit 1 (COI) gene confirmed its identity as PSHB, and specifically one of the invasive haplotypes of the beetle. The associated fungus *F. euwallaceae* was identified based on phylogenetic analysis of elongation factor (*EF 1-α*) sequences. Koch's postulates have confirmed the pathogenicity of fungal isolates to *P.* x *acerifolia*. This is the first report of PSHB and its fungal symbiont causing Fusarium dieback in South Africa. This report also represents the first verified case of a damaging invasive forest pest detected in a sentinel planting project, highlighting the importance of such studies. Given the potential impact these species present to urban trees, native biodiversity and agriculture, both the PSHB and its fungal symbiont should be included in invasive species regulations in South Africa.

Keywords Invasive pest · Fusarium dieback · *Euwallacea* nr. *fornicatus* · International Plant Sentinel Network

Introduction

Worldwide, there is a growing list of damaging invasive forest pests, the introduction of which has largely been precipitated by international trade and the intentional movement of plant material (Santini et al. 2013; Liebhold et al. 2012). Many of these introductions involve organisms that were not problematic in their native range, or were unknown to science prior to their arrival in a new environment. Consequently, they could not have been regulated against, or detected and stopped at checkpoints (Eschen et al. 2015; Wingfield et al. 2015; Brasier 2008). In response to this growing threat, there has been a move towards the use of 'sentinel plantings', where exotic species growing outside of their natural range are utilised to provide an early warning system to identify new pest and pathogen risks to plants (Vettraino et al. 2015; Roques et al. 2015). Botanical gardens and arboreta host a large range of exotic plant collections in diverse regions around the world, thus presenting a unique opportunity for sentinel research. The International Plant Sentinel Network (IPSN) was launched in 2013 as a platform to coordinate information exchange and provide support for sentinel plant research within botanical gardens and arboreta (Barham 2016; Britton et al. 2010). In addition to their value in identification of novel host-pest

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interactions, when they are adjacent to high-risk sites such as ports, botanical gardens and arboreta can also provide opportunities to detect damaging invasive forest pests during their initial stages of establishment (Burgess and Wingfield 2017; Tubby and Webber 2010; Paap et al. 2017).

The polyphagous shot hole borer (PSHB), an ambrosia beetle (Coleoptera: Curculeonidae: Scolytinae) native to Asia, has emerged as an important invasive pest in Israel and in California in the United States. In these countries, it is causing significant and costly damage to urban trees, as well as presenting a major threat to the avocado industries. As adult female beetles burrow into trees to establish brood galleries, they introduce the symbiotic fungus *Fusarium euwallaceae*, which colonises gallery walls, becoming a food source for developing larvae and adult beetles (Eskalen et al. 2012; Mendel et al. 2012). The fungus then invades tree vascular tissue, causing cambial necrosis, branch dieback and death of a broad range of trees (Eskalen et al. 2013). The PSHB is one of three cryptic species in the *Euwallacea fornicatus* species complex, the taxonomy of which remains to be resolved.

In 2016 a project was established in South Africa to improve surveillance and identification of new and emerging pest risks by using botanical gardens and arboreta as sentinel sites for tree health monitoring. During routine surveys monitoring tree health in the KwaZulu-Natal National Botanical Gardens (KZN NBG), Pietermaritzburg, South Africa, *Platanus* x *acerifolia* (London Plane) trees showing symptoms of ambrosia beetle attack were observed. Removal of the bark and cambium exposed galleries with lesions, from which wood material was sampled. Additionally, infested branch material was collected from which PSHB and its fungal symbiont *F. euwallaceae* were identified. We report here on the first record of PSHB and *F. euwallaceae* causing Fusarium dieback in South Africa.

Materials and methods

Specimen collection and fungal isolation

Whilst undertaking tree health monitoring surveys in the KwaZulu-Natal National Botanical Gardens (KZN NBG), Pietermaritzburg, South Africa, *Platanus* x *acerifolia* trees showing symptoms of ambrosia beetle attack were observed (Fig. 1a and b). A sterilised chisel was used to remove bark and cambium from suspected beetle entry holes, with symptomatic tissues frequently observed at a depth beyond the cambium (Fig. 1c). An infested branch section was removed from one of the trees and double-bagged with heavyweight trash bags for transport to the laboratory. The branch was split to check for gallery formation, presence of adult beetles, eggs, larvae and pupae (Figs. 1d and 2). Fungal isolates (Table 1) were obtained from symptomatic material after surface disinfestation by briefly flaming with 80% ethanol, and plating on 2% Malt Extract Agar (MEA, Biolab, South Africa).

DNA isolation, amplification and sequencing

Fungal isolates

DNA was extracted from fresh mycelium scraped from actively growing cultures as described in Duong et al. (2012). The elongation factor 1-α region was amplified using the primer pair EF1 and EF2 and the PCR thermocycling conditions described in O'Donnell et al. (2010). Each 25 μl PCR reaction contained 2 μl template DNA, 2.5 μl of 10× PCR buffer, 200 μM of each dNTP, 0.2 μl of both the forward and reverse primer, 1 U FastStart Taq DNA polymerase (Roche), 2.5 mM $MgCl_2$, and 16.4 μl nuclease free water. PCR products were purified by adding 8 μl ExoSap solution (1 U Exonuclease 1, 1 U Shrimp alkaline phosphatase) to 23 μl PCR product and incubating the mixture at 37 °C for 15 min and then at 80 °C for another 15 min. Sequencing and contig assembly was done for both the forward and reverse primers as described by Duong et al. (2012). Sequences derived in this study were added to GenBank and accession numbers are provided in Table 1.

Beetles

Genomic DNA was extracted and precipitated from beetle samples using a modified version of the method described in Duong et al. (2013). Working concentrations for PCR amplification were prepared by diluting 2 μl of the concentrated DNA solution in 8 μl Tris-HCl (10 mM, pH 8.0). The COI region was amplified using the primer pair LCO1490 and HCO2198 and the PCR thermocycling conditions as described in Hebert et al. (2003). Each 25 μl PCR reaction contained 2 μl template DNA, 2.5 μl of 10× PCR buffer, 200 μM of each dNTP, 0.2 μl of both the forward and reverse primer, 1 U FastStart Taq DNA polymerase, 2.5 mM $MgCl_2$, and 16.4 μl nuclease free water. Products were purified by adding 8 μl ExoSap solution (1 U Exonuclease 1, 1 U Shrimp alkaline phosphatase) to 23 μl PCR product and incubating the mixtures at 37 °C for 15 min and then at 80 °C for another 15 min. Sequencing and contig assembly was done for both the forward and reverse primers using the method described by Duong et al. (2012).

Phylogenetic analysis

Data sets were compiled in MEGA 7.0.26 (Kumar et al. 2016) using sequences generated in this study together with sequences from previous studies obtained from GenBank. Also included in the beetle sequence data set was a single sequence (BOLD: ETKC270–13) obtained from a beetle from

Fig. 1 a-b. external symptoms of polyphagous shot hole borer attack on *Platanus* x *acerifolia*; c. removal of bark and cambial tissue exposing symptoms caused by fungal colonisation associated with beetle entry hole; d. longitudinal section through branch showing internal symptoms of discolouration around beetle gallery

an unknown host near Durban, South Africa, during a 'Barcode of Life' project (www.barcodeoflife.org). DNA sequence alignments were done using the online version of MAFFT 7 (Katoh and Standley 2013).

Pathogenicity tests

Two isolates of *F. euwallaceae* (Table 1) were used in pathogenicity tests. Isolates were grown on 2% MEA for 5 days before inoculation of 300 mm long detached healthy woody shoots of *P.* x *acerifolia*. Xylem tissue was excised from the centre of each stem (mean diameter 9.5 mm ± 0.2 mm) with a 3 mm cork borer. A 3 mm diameter colonised agar plug was taken from the leading edge of each growing culture, placed onto the freshly wounded tissue with the mycelium face down, and the inoculated area wrapped with Parafilm. Clean agar disks were used to inoculate stems as a negative control. Stem ends were dipped in wax to prevent desiccation, and stems were incubated in moist chambers at 25 ± 1 °C for 2 weeks. At harvest, the extent of vascular discolouration was assessed and re-isolations were made to recover the inoculated fungi. The experiment was arranged in a randomised design with 10 replications per isolate and conducted twice.

Statistical analysis

Lesion length data from pathogenicity tests were checked for normality, and significant differences among mean values were assessed by analysis of variance (ANOVA) and post hoc Least Significant Difference (LSD) test at $\alpha = 0.05$.

Fig. 2 Female polyphagous shot hole borer (*Euwallacea* nr. *fornicatus*)

Statistical analyses were performed using XLSTAT software (Addinsoft, Paris, France).

Results

Fungal isolation and identification

Isolates resembling *Fusarium* spp. were obtained from symptomatic tissues up to 3 cm from beetle entry points (Table 1). DNA sequences of the EF1-α gene region from these isolates were identical to those of the ex-holotype of *F. euwallaceae* from *Persea americana* (avocado) in Israel, as well as isolates from a wide variety of host trees in California, USA (Supplementary Figure S1). Fungal sequences generated in this study have been deposited in GenBank (Table 1).

Recovery and identification of beetles

Splitting of the branch section revealed clear evidence of gallery formation (Fig. 1d). However, galleries were only up to 3 cm long with no branching. Evidence of reproduction was not apparent, with eggs and/or beetle larvae absent. Two adult female beetles were located within the galleries. Based on morphological characters and using the taxonomic keys by Rabaglia et al. (2006), these were identified as *Euwallacea* nr. *fornicatus.* The CO1 sequence was obtained for one of the beetles (GenBank accession number MG642816) and showed a 100% match (Supplementary Figure S2) with haplotype H33 of the PSHB clade of the *E. fornicatus* species complex as defined by Stouthamer et al. (2017). Other specimens presenting haplotype H33 came from Durban, South Africa, collected during Barcode of life project, *Ricinus communis* (castorbean) from Vietnam and California, and *Persea americana* (avocado) in Israel.

Table 1 *Fusaium euwallaceae* isolates from *Platanus* x *acerifolia* in the KwaZulu-Natal National Botanical Gardens (Pietermaritzburg, South Africa) considered in this study and GenBank accession numbers

Species	CMW culture number[a]	GenBank accession number (EF1-α)
F. euwallaceae	CMW50555[b]	MG642810
F. euwallaceae	CMW50556	MG642811
F. euwallaceae	CMW50557	MG642812
F. euwallaceae	CMW50558	MG642813
F. euwallaceae	CMW50559[b]	MG642814
F. euwallaceae	CMW50560	MG642815

[a] CMW = culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa

[b] Isolates used in pathogenicity trial

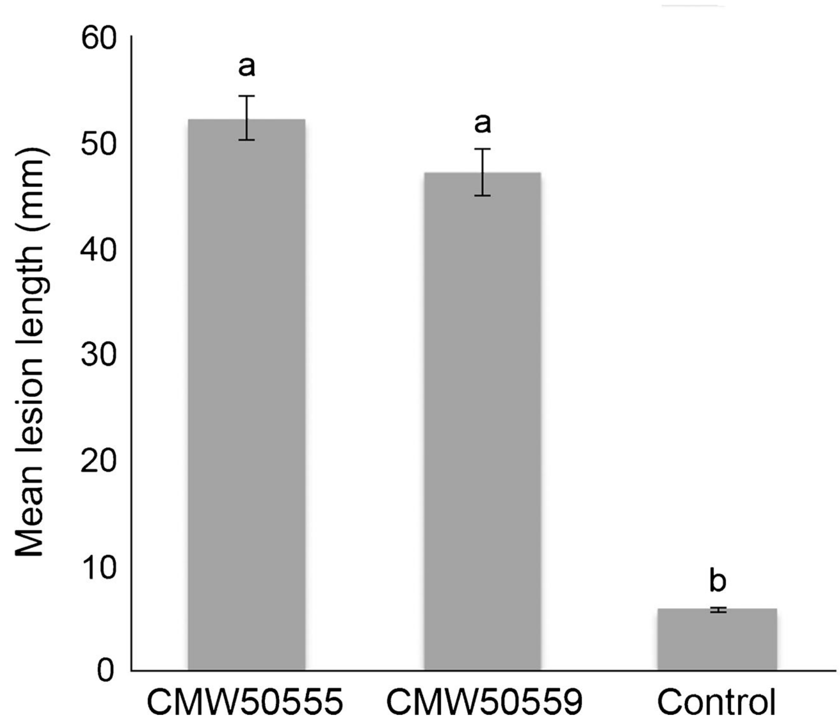

Fig. 3 Mean lesion length (mm) on *Platanus* x *acerifolia* excised stems 2 weeks after inoculation with *Fusarium euwallaceae* isolates CMW50555 and CMW50559. Vertical lines represent standard error of mean. Values with the same letter above the bar do not differ significantly at $p = 0.05$ according to LSD test

Pathogenicity tests

Both isolates of *F. euwallaceae* colonised healthy inoculated excised stems of *P.* x *acerifolia* (Fig. 3). The fungus was recovered from all of the inoculated stems. Lesion lengths did not vary significantly between trials and data were consequently pooled. Mean lesion lengths were significantly different between fungal inoculation treatments and controls ($P < 0.0001$), although fungal isolates did not differ significantly in their ability to produce lesions. The fungus was successfully re-isolated from the lesions.

Discussion

In response to the growing threat posed by invasive forest pests, there has been a move towards the use of sentinel plantings to identify new host-pest interactions (Tomoshevich et al. 2013; Roques et al. 2015; Vettraino et al. 2015). Whilst undertaking tree health monitoring as part of a sentinel research project established in South Africa, we detected the damaging invasive forest pest PSHB. This study provides the first report of PSHB and its fungal symbiont, *F. euwallaceae,* causing Fusarium dieback on trees in South Africa. It also represents the first case of a damaging invasive forest pest being detected through a sentinel research project, highlighting the value and significance of investing in such research.

Whilst there was no evidence of beetle reproduction on the *P.* x *acerifolia* branch collected, other *Platanus* spp. including *P. occidentalis, P. orientalis* and *P. racemosa* have been found to be reproductive hosts of the insect (Eskalen et al. 2013; Mendel et al. 2017). The lack of observed reproduction in the collected sample may relate to branch diameter and distance from the point of branching. Mendel et al. (2017) found reproductive galleries (in avocado) were largely found at the base of the branches, close to the branching points. The branch section obtained during the present study was several meters away from the branching point, as branch diameter and accessibility precluded removal at the branch junction.

In addition to *F. euwallaceae, Graphium euwallaceae* and *Paracremonium pembeum* have been recorded as symbiotic fungal species associated with PSHB (Lynch et al. 2016). Whilst the latter two species were not observed growing from necrotic host tissue in the current study, further isolations from galleries and beetles may identify their presence.

In a recent study, Stouthamer et al. (2017) included a single sequence from an unidentified coleopteran specimen obtained in a Barcode of Life project (BOLD: ACC9773, ETKC270–13) from Durban, South Africa, in their phylogeny of the *E. fornicatus* species complex. The specimen was identified as haplotype H33 of the PSHB. The beetles collected during the current study from Pietermaritzburg (50 km NW of the BOLD trapping location) were identified as this same haplotype. This haplotype has been identified as one of the four invasive haplotypes in the *E. fornicatus* complex (Stouthamer et al. 2017). The BOLD collection was made in 2012, suggesting the beetle has likely been present but undetected in the region for some years.

The discovery of PSHB and associated Fusarium dieback in South Africa is significant and of major concern. The beetle is native to Asia, and appears to be a generalist species, with the appellation 'polyphagous' referring to the broad range of trees attacked (Umeda et al. 2016). PSHB has had a major negative impact on trees in urban landscapes, forests and fruit production (particularly avocado) where it has invaded in California and Israel, with infestations of susceptible trees resulting in high levels of mortality (Mendel et al. 2017; Eskalen et al. 2013). Eskalen et al. (2013) examined the host range of the beetle-fungus complex in two heavily infested botanical gardens in Los Angeles County, determining that of 335 tree species observed, 207 (representing 58 plant families), showed symptoms of attack by PSHB. These included several species native to southern Africa, including *Cussonia spicata* (cabbage tree), *Calpurnia aurea* (common calpurnia), *Diospyros lycioides* (monkey plum), *Erythrina humeana* (dwarf coral tree), *Erythrina lysistemon* (common coral tree), *Schotia brachypetala* (huilboerboon), *Melianthus major* (honey flower, kruidjie-roer-my-nie), *Cunonia capensis* (red alder), *Nuxia floribunda* (forest elder) and *Bauhinia galpinii* (red orchid bush). Most of these species showed some level of susceptibility to Fusarium dieback, except the last three that were infested by the beetle but did not develop disease. Based on the survey of Eskalen et al. (2013), several commercial crop trees that are planted in South Africa, such as *Persea americana* (avocado), *Macadamia integrifolia* (macadamia nut), *Carya illinoinensis* (pecan), *Prunus persica* (peach), *Citrus sinensis* (orange) and *Vitis vinifera* (grapevine), are susceptible to PSHB infestation and Fusarium dieback. Eskalen et al. (2013) also listed as susceptible several trees that while exotic to South Africa, are planted as ornamentals, including maple, holly, wisteria, oak and Camellia.

Many of these potential hosts of the PSHB are present in the KwaZulu-Natal region, including the widespread woody weed, castor bean (*Ricinus communis*). This species is an important reproductive host in California (Eskalen et al. 2013) and could potentially act as a corridor for invasion across urban, agricultural and native ecosystem interfaces (Umeda et al. 2016). PSHB and Fusarium dieback represent a significant new threat to trees in urban and natural areas, as well as to avocado orchards in South Africa. Both should be considered for listing under South Africa's National Environmental Management: Biodiversity Act: Alien and Invasive Species (NEM:BA AIS) Regulations, and immediate surveys to determine the extent of PSHB presence in South Africa should be undertaken to assist in the development of management actions to reduce the risk of spread.

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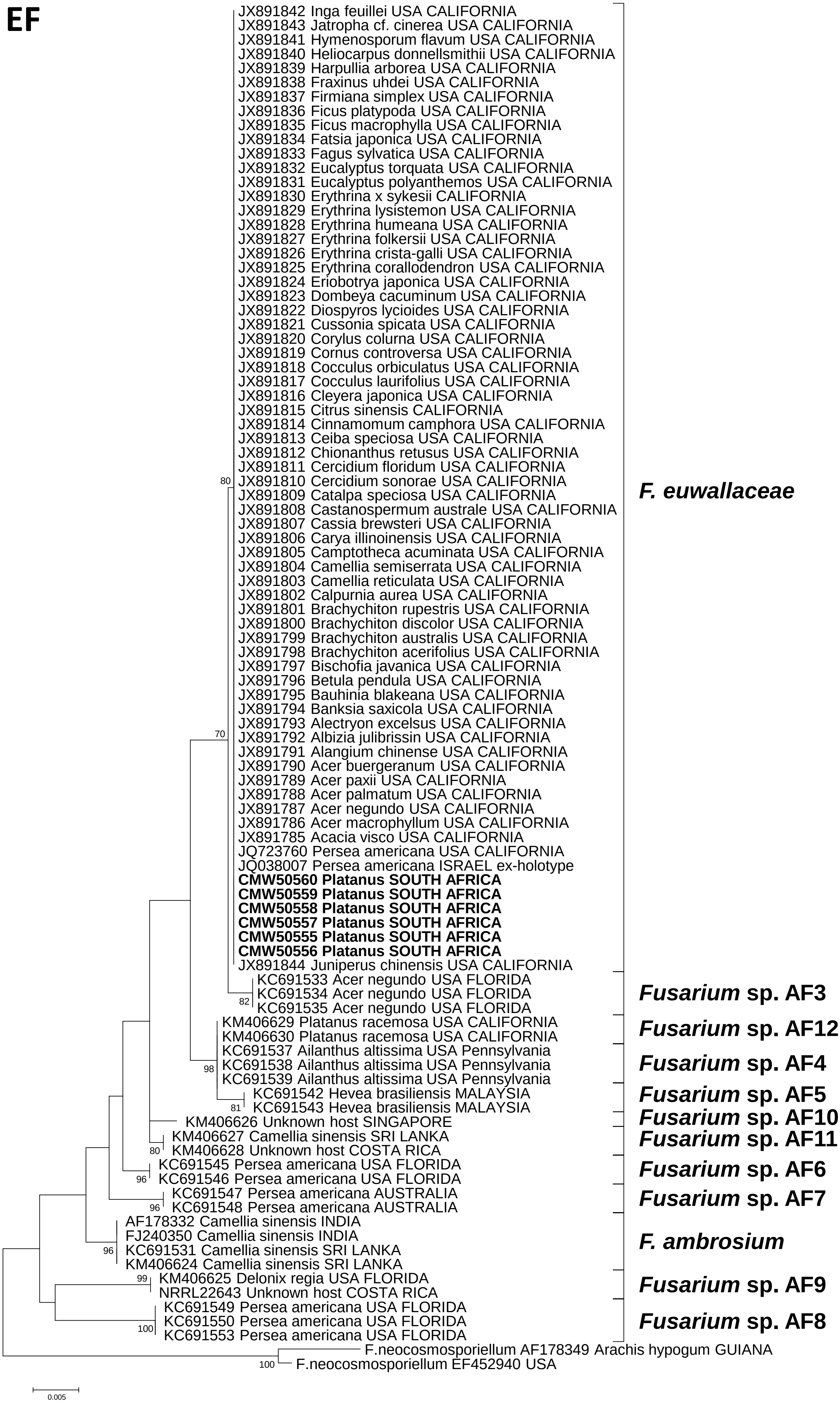
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JX891842 Inga feuillei USA CALIFORNIA
JX891843 Jatropha cf. cinerea USA CALIFORNIA
JX891841 Hymenosporum flavum USA CALIFORNIA
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JX891819 Cornus controversa USA CALIFORNIA
JX891818 Cocculus orbiculatus USA CALIFORNIA
JX891817 Cocculus laurifolius USA CALIFORNIA
JX891816 Cleyera japonica USA CALIFORNIA
JX891815 Citrus sinensis CALIFORNIA
JX891814 Cinnamomum camphora USA CALIFORNIA
JX891813 Ceiba speciosa USA CALIFORNIA
JX891812 Chionanthus retusus USA CALIFORNIA
JX891811 Cercidium floridum USA CALIFORNIA
80
JX891810 Cercidium sonorae USA CALIFORNIA
JX891809 Catalpa speciosa USA CALIFORNIA
JX891808 Castanospermum australe USA CALIFORNIA
JX891807 Cassia brewsteri USA CALIFORNIA
JX891806 Carya illinoinensis USA CALIFORNIA
JX891805 Camptotheca acuminata USA CALIFORNIA
JX891804 Camellia semiserrata USA CALIFORNIA
JX891803 Camellia reticulata USA CALIFORNIA
JX891802 Calpurnia aurea USA CALIFORNIA
JX891801 Brachychiton rupestris USA CALIFORNIA
JX891800 Brachychiton discolor USA CALIFORNIA
JX891799 Brachychiton australis USA CALIFORNIA
JX891798 Brachychiton acerifolius USA CALIFORNIA
JX891797 Bischofia javanica USA CALIFORNIA
JX891796 Betula pendula USA CALIFORNIA
JX891795 Bauhinia blakeana USA CALIFORNIA
JX891794 Banksia saxicola USA CALIFORNIA
JX891793 Alectryon excelsus USA CALIFORNIA
70
JX891792 Albizia julibrissin USA CALIFORNIA
JX891791 Alangium chinense USA CALIFORNIA
JX891790 Acer buergeranum USA CALIFORNIA
JX891789 Acer paxii USA CALIFORNIA
JX891788 Acer palmatum USA CALIFORNIA
JX891787 Acer negundo USA CALIFORNIA
JX891786 Acer macrophyllum USA CALIFORNIA
JX891785 Acacia visco USA CALIFORNIA
JQ723760 Persea americana USA CALIFORNIA
JQ038007 Persea americana ISRAEL ex-holotype
CMW50560 Platanus SOUTH AFRICA
CMW50559 Platanus SOUTH AFRICA
CMW50558 Platanus SOUTH AFRICA
CMW50557 Platanus SOUTH AFRICA
CMW50555 Platanus SOUTH AFRICA
CMW50556 Platanus SOUTH AFRICA
JX891844 Juniperus chinensis USA CALIFORNIA
F. euwallaceae
KC691533 Acer negundo USA FLORIDA
KC691534 Acer negundo USA FLORIDA
82
KC691535 Acer negundo USA FLORIDA
Fusarium sp. AF3
KM406629 Platanus racemosa USA CALIFORNIA
KM406630 Platanus racemosa USA CALIFORNIA
Fusarium sp. AF12
KC691537 Ailanthus altissima USA Pennsylvania
98
KC691538 Ailanthus altissima USA Pennsylvania
KC691539 Ailanthus altissima USA Pennsylvania
Fusarium sp. AF4
KC691542 Hevea brasiliensis MALAYSIA
81
KC691543 Hevea brasiliensis MALAYSIA
Fusarium sp. AF5
KM406626 Unknown host SINGAPORE
Fusarium sp. AF10
KM406627 Camellia sinensis SRI LANKA
80
KM406628 Unknown host COSTA RICA
Fusarium sp. AF11
KC691545 Persea americana USA FLORIDA
96
KC691546 Persea americana USA FLORIDA
Fusarium sp. AF6
KC691547 Persea americana AUSTRALIA
96
KC691548 Persea americana AUSTRALIA
Fusarium sp. AF7
AF178332 Camellia sinensis INDIA
FJ240350 Camellia sinensis INDIA
96
KC691531 Camellia sinensis SRI LANKA
KM406624 Camellia sinensis SRI LANKA
F. ambrosium
99
KM406625 Delonix regia USA FLORIDA
NRRL22643 Unknown host COSTA RICA
Fusarium sp. AF9
KC691549 Persea americana USA FLORIDA
100
KC691550 Persea americana USA FLORIDA
KC691553 Persea americana USA FLORIDA
Fusarium sp. AF8
F.neocosmosporiellum AF178349 Arachis hypogum GUIANA
100
F.neocosmosporiellum EF452940 USA
0.005

CO1
ETKC270-13 BOLD:ACC9773 SOUTH AFRICA KZN
F0005 Platanus SOUTH AFRICA KZN
JX912724 H33 Ricinus communis USA CALIFORNIA
KM406722 H33 Persea americana ISRAEL
KU727021 H33 Ricinus communis VIETNAM
KU727022 H34 Ricinus communis VIETNAM
JX912723 H35 Ricinus communis USA CALIFORNIA
KM406726 H35 Quercus robur USA CALIFORNIA
KM406727 H35 Acer USA CALIFORNIA
KU727023 H35 Ricinus communis USA CALIFORNIA
KU727020 H32 Ricinus communis VIETNAM
KU727024 H36 Persea americana TAIWAN
KU727025 H37 Persea americana TAIWAN
KU727026 H38 Persea americana TAIWAN
KU727027 H39 Unknown host JAPAN
KU727030 H42 Oreocnide pedunculata TAIWAN
KU727028 H40 Oreocnide pedunculata TAIWAN
KU727029 H41 Oreocnide pedunculata TAIWAN
KU727018 H30 Unknown host TAIWAN
KU727013 H25 Acer buergerianum CHINA Yunnan
KU727019 H31 Cinnamomum cassia VIETNAM
KU727015 H27 Acacia mangium VIETNAM
KU727016 H28 Persea americana Thailand
KU727017 H29 Platanus sp. CHINA Yunnan
KU727011 H23 Acacia mangium VIETNAM
KU727012 H24 Acacia mangium VIETNAM
KU727014 H26 Acacia mangium VIETNAM
KU727010 H22 Ricinus communis TAIWAN
KU727005 H17 Unknown host JAPAN
KU727004 H16 Unknown host JAPAN
KU727006 H18 Unknown host TAIWAN
KU727009 H21 Unknown host TAIWAN
KU727007 H19 Unknown host TAIWAN
KU727008 H20 Unknown host TAIWAN
HM064086 E.xanthopus TAIWAN
KU727034 E.xanthopus trap SOUTH AFRICA Eastern Cape
KU727032 Euwallacea sp. TAIWAN
KU727033 Euwallacea sp. Acacia UGANDA
KU726991 H1 Cinnamon SINGAPORE
KU726993 H3 Citrus sp. PNG
KU726992 H2 Unknown host AUSTRALIA
KM406729 H2 Unknown host AUSTRALIA
KM406733 H4 Camellia sinensis SRI LANKA
KU726994 H4 Camellia sinensis SRI LANKA
KJ866509 H5 Camellia sinensis INDIA
KJ866508 H6 Camellia sinensis INDIA
KU726995 H7 Durio zibethinus THAILAND
KM406728 H8 Persea americana USA FLORIDA
KU726996 H8 Persea americana USA FLORIDA
KM406731 H8 Persea americana USA FLORIDA
KU726997 H9 Durio sp. THAILAND
KU726998 H10 Durio sp. THAILAND
KU726999 H11 Unkown host THAILAND
KU727000 H12 Durio sp. THAILAND
KU727001 H13 Durio zibethinus THAILAND
KU727002 H14 Durio zibethinus THAILAND
KU727003 H15 Unknown host THAILAND
KU727039 E.andamanensis THAILAND
HM064083 E.andamanensis THAILAND
KU727041 E.interjectus VIETNAM
KM406716 E.interjectus USA FLORIDA
KP313802 E.interjectus USA FLORIDA
KM406719 E.validus USA MARYLAND
KP313803 E.validus USA OHIO
HM064087 E.validus USA
HM064084 E.andamanensis/talumalai PNG
KU727040 E.andamanensis/talumalai PNG
KP313799 E.andamanensis PNG
KU727038 Euwallacea sp. VIETNAM
KU727037 E.similis VIETNAM
KU727036 E.similis THAILAND
KU727035 E.similis THAILAND
HM064085 E.subemarginatus BORNEO
GU808698 E.russulus PNG
GU808707 Xylosandrus compactus GHANA
KU727031 Xylosandrus compactus VIETNAM
Haplotype 33
Haplotype 35
PSHB
Haplotype 20
KSHB
E. xanthopus
Haplotype 8
TSHB
Euwallacea fornicatus species complex