Action D1.
Analyses of data and samples,
evaluation and optimization of techniques

Deliverable: Final list of fungi associated to *Xylosandrus* sp.

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Prepared by: Carmen Morales-Rodríguez

With support of: Cécile Robin and Andrea Vannini

Revision history

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1	07/12/2021	Carmen Morales-Rodriguez (UNITUS)	X. germanus is included. Revision of fungal list from isolation methods
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Summary

Insects, like many other organisms, live in association with many fungal symbionts, which can have a positive (i.e., mutualistic), negative (i.e., parasitic) or neutral (i.e., commensalistic) impact on their host's fitness. Symbiotic fungi associated with Ambrosia beetles contribute with insect damage to the impact to invaded environments. Also of providing food for the insect development stages, some of the Ambrosia beetle symbiotic fungi are severe pathogens of plant hosts. The most relevant example is *Xyleborus glabratus* carrying the fungus *Raffaela lauricola* cause of lethal vascular wilt of avocado, that is devastating the plantations in South-eastern United States. Thus, monitoring of alien fungi introduced through specific pathways (mostly trading of living plants), their identification and determination of pathogenicity behaviour is essential to design and apply prevention and mitigation quarantine measures. This deliverable describes the fungal community isolated from *X. compactus*, *X. crassiusculus* and *X. Germanus*.

1. Bark beetle' and obligate symbiosis with fungi

Bark beetle is both a taxonomic and ecological designation. In the taxonomic sense, bark beetles are all species in the weevil subfamily Scolytinae, including species that do not consume bark. In the ecological sense, bark beetles are species of Scolytinae whose larvae and adults live in and consume phloem of trees and other woody plants. They are not obligatory associated with fungal symbionts, while ambrosia beetles are obligately associated with nutritional fungal symbionts. Obligate symbiosis with fungi is present in at least 11 independent scolytinae and platypodine groups. Ambrosia beetles are therefore not monophyletic, and the name is not a taxonomic designation. Ambrosia beetles are derived from bark beetles (Coleoptera: Curculionidae: Scolytinae). Bark beetles colonize and consume phloem, a tissue that is more nutrient-rich than wood. Bark beetles, like ambrosia beetles, are also often associated with fungal symbionts, usually ascomycotan and rarely basidiomycotan fungi, and the intensity of association is more variable, ranging from facultative to obligate (You L, 2015).
One of the most common symbioses in any forest ecosystem occurs between wood-boring insects and fungi.

This kind of symbiosis between Ambrosia beetles (Coleoptera: Curculionidae: Scolytinae and Platypodinae) and ambrosia fungi is ideal for studying many symbiosis-related questions. One reason is the diversity of ambrosia beetles which represent about 3000 species of wood-boring weevils that repeatedly evolved obligate symbioses with nutritional fungi possibly up to 16 times. Another reason is the easy manipulation of the symbiosis. Although the two partners require each other to complete their life cycle, they are perfectly separable in vitro. The beetles' transport-specific fungal symbionts from their natal galleries to newly established galleries in a storage organ termed a mycangium, but both can be kept in the laboratory on artificial media. Furthermore, their importance needs to be taken into consideration as in recent years, several ambrosia beetle-fungus symbioses have developed outbreaks causing significant economic and ecological damages. Unquestionably, it is important to understand the interactions between the beetle and the fungus has immediate scientific, economic and ecological implications (You Li1, 2018). Moreover, like many insects, ambrosia beetles may carry commensalist fungi on their body.

2. *Xylosandrus crassiusculus* (motschulsky)

Xylosandrus crassiusculus (Asian ambrosia beetle or granulate ambrosia beetle) it is a highly polyphagous pest of woody plants of Asian origine and has been spread most probably with trade of plants and wood. In Africa it arrived hundreds of years ago while in the last few years it has been introduced to at least 14 African countries, 25 USA states, 3 countries of Central America, 2 South American countries, 6 countries of Oceania and 2 European countries (EPPO 2015, Fletchmann and Atkinson 2016), Italy and France, recently adding Spain (Gallego et al., 2017). Since the 1970s it has become a pest of fruit tree orchards and ornamental tree nurseries in the USA (EPPO Alert-list). It was first found in Europe in 2003, in cross-vane traps set up in Tuscany (Livorno, NW Italy) where no specific control measures were adopted (Pennacchio et al. 2003; EPPO 2015). Later, carob trees attacked by *X. crassiusculus* were found in in orchards in Central-North Italy and in gardens of nearby Liguria (Alassio and Pietraligure, NW Italy) in 2007 and 2008, and in NE Italy in Veneto (EPPO 2015) and Friuli Venezia Giulia (2015, personal observation of Massimo Faccoli). Maybe via Liguria, in 2014 the species arrived in SE France and in the Spanish Valencia Region.

Adults are small dark reddish brown scolytids (female: 2-3 mm long, males: 1.5 mm). Larvae are white, legless, C-shaped with a well-developed capsule, and cannot be easily distinguished from other scolytids. Populations essentially contain females (1:10 male-female ratio). Adult males do not fly and remain inside the galleries. Also *X. crassiusculus* is an inbreeding species (females mate with their brothers).

When females emerge, they leave infested plants and fly to new hosts. They start to bore a tunnel (round entrance hole of 2 mm diameter) with a brood chamber and one or more branches into the sapwood (and sometimes the heartwood). Eggs are laid in the brood chamber. Larvae have a length about 3.5 mm. and hatch and feed on the symbiotic fungus growing inside the galleries (Gardner, 1934, CABI Factsheet).

2.1. List of fungi associated to *Xylosandrus crassiusculus*

The fungal isolation and detection by High-throughput sequencing (HTS) were done according to the protocol described on SAMFIX Deliverable: *Fast routine protocol for detection of fungal symbiotic community associated with trapped Xylosandrus*. *Briefly, for the isolation* the insects are crumbled in PBS and serial dilutions are plated on PDA. Different colonies are subcultured in new PDA plates. Morphotype designations are confirmed by ITS DNA sequencing and compared with sequences available at NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The fungi isolated from *X. crassiusculus* are on Table 1. For fungal detection by HTS, total DNA from insect is extracted. The ITS1 region is amplified with a dual indexing primer using the tagged primer pair ITS1F (5'-xxxxCTYGGTCATTTAGAGGAAGTAA-3') and ITS2 (5-xxxxGCHRCGTTCTTCATCGDTGC-3'), where xxx represents the barcoding key. Amplicons are purified and quantified. Finally, paired-end sequencing (2 x 300 bp) is carried out on an Illumina MiSeq sequencer.

Table 1. List of fungi isolated from *Xylosandrus crassiusculus*

SPECIES	PHYLUM	ORDER	BODY PART	FUNCTIONAL GUILD	OCCURANCE	REPORTED HOST/SUBSTRATE	REFERENCE
Acremonium camptosporum	A	Hypocreales	Mycangia	Plant pathogen	Asia	White mold spots	Sun et al., 2019
Acremonium roseolum	A	Hypocreales	Abdomen	Unassigned	Asia, South America	*Cryptomeria japonica* and Manihot esculenta 6	https://nt.ars-grin.gov/fungaldatabases
Alternaria alternata	A	Pleosporales	Abdomen, head, mycangia, external	Plant pathogens-Saprotrophs	Widespread	Wide-host range	Feng, Zheng, 2007
Alternaria infectoria	A	Pleosporales	External	Plant pathogen	Widespread in temperate regions.	Substrate: Kernels, leaves. Disease: Black point disease.	https://nt.ars-grin.gov/fungaldatabases
Ambrosiella roeperi	A	Microascales	Mycangia	Symbiont	Widespread	Fungal symbiont of *X. crassiusculus*	Harrington et al., 2014
Bipolaris sorokiniana	A	Pleosporales	Abdomen	Plant pathogen	Cosmopolitan.	Leat spot, seedling blight, and root rot. Primarily on Poaceae but also numerous and diverse other hosts.	https://nt.ars-grin.gov/fungaldatabases

Biscogniauxia nummularia	A	Xylariales	Abdomen	Saprotrophs-Plant pathogens	Cosmopolitan.	Fagus spp. (Fagaceae) and other hardwoods. Usually saprotrophic; also pathogenic, causing canker	https://nt.ars-grin.gov/fungaldatabases
Cladosporium cladosporioides	A	Capnodiales	Abdomen, head, external	Saprotrophs-Hyperparasitic	Cosmopolitan	Multiple genera in multiple families; Leaves, seeds, inflorescences, often dead plant material; Causing leaf spot and blight, flower blight, scab, sooty mold.	Barkat et al., 2016
Cladosporium herbarum	A	Capnodiales	External	Saprotrophs	Cosmopolitan	Multiple genera in multiple families. Plant material and other organic substrates. Various spots and rots.	Bensch et al., 2012
Clonostachys rosea	A	Hypocreales	Mycangia	Plant pathogens-Saprotrophs	Cosmopolitan	Various plant parts both living and newly killed,associated with bark beetle galleries	Nygren et al., 2018
Crinipellis scabella	B	Agaricales	Mycangia	Unassigned	Widespread	On soil	Antonin et al., 2009
Cryphonectria parasitica	A	Diaporthales	mycangia	Plant pathogen	Asia, Europe, North America	*Castanea* spp., *Fagus sylvatica*, *Quercus* spp. (Fagaceae).	https://nt.ars-grin.gov/fungaldatabases
Didymella glomerata	A	Pleosporales	External	Plant pathogens-Saprotrophs	Cosmopolitan	Various plant genera. Opportunistic pathogen. Found in association with blights, leaf spots, fruit rots.	https://nt.ars-grin.gov/fungaldatabases
Epicoccum nigrum	A	Pleosporales	Testa	Plant pathogens-Saprotrophs	Cosmopolitan	Diverse host, associated with decline 7	https://nt.ars-grin.gov/fungaldatabases
Fusarium solani	A	Hypocreales	Abdomen, head, mycangia, external	Plant pathogen	Cosmopolitan	Broad host range; associated with ambrosia beetles	Sharma and Marques, 2018
Geosmithia pallida	A	Hypocreales	Abdomen, mycangia, external	Plant pathogen	Widespread	Associated with bark beetle *Pseudopithyophthorus pubipennis*; Hosts: Quercus spp. (Fagaceae), Prunus spp., Malus (Rosaceae); Causing Foamy bark canker, dieback, death	Kolarik et al., 2017
Hyphopichia pseudoburtonii	A	Sccharomycetales	Abdomen, mycangia	Unassigned	Widespread	On soil	Groenewald and Smith 2010

Paraconiothyrium archidendri	A	Xylariales	Abdomen, head, mycangia, external	Plant pathogen	Asia (Myanmar)	Leaf spot on *Pithecellobium bigeminum* (Fabaceae).	Verkley et al., 2014
Penicillium adametzioides	A	Eurotiales	Mycangia	Plant pathogen	Europe, Asia	On Vitis vitifera	Lorenzoni et al., 2018; Kobayashi 2007
Penicillium brevicompactum	A	Eurotiales	Abdomen	Plant pathogen	Cosmopolitan	Rots on numerous host	https://nt.ars-grin.gov/fungaldatabases
Penicillium expansum	A	Eurotiales	Abdomen, head, external	Plant pathogen-Saprotrophs	Cosmopolitan	Numerous hosts.Fruit, decaying vegetation, seeds, etc. Causing Blue mold, fruit rot, postharvest decay.	https://nt.ars-grin.gov/fungaldatabases
Penicillium glabrum	A	Eurotiales	Testa	Saprotrophs	Cosmopolitan	Numerous substrates; Numerous hosts; causing post harvest fruit rot	Duduk et al., 2017
Penicillium sumatrense	A	Eurotiales	Testa	Plant pathogen	Asia	Blue mold on diverse host	Liu and Lu 2018
Peniophora quercina	B	Russulales	Abdomen, mycangia	Saprotroph	Temperate northern hemisphere	Wood decay	Boddy and Rayner 1984
Peroneutypa scoparia	A	Xylariales	Abdomen	Plant pathogen	Europe, South America	Chlorosis, dieback, short internodes, mortality; *A.deliciosa*, *R. pseudoacacia*, *A. pseudoplatanus*, *V.corymbosum*	https://nt.ars-grin.gov/fungaldatabases
Pestalotiopsis vismiae	A	Xylariales	Mycangia	Plant pathogen	Asia (China, India), North America	Numerous hosts.Substrate: Trunk, bark, leaves, petioles, roots. Disease Note: Bark cracking, lesions; trunk disease.	https://nt.ars-grin.gov/fungaldatabases
Pithomyces chartarum	A	Pleosporales	Head, external	Plant pathogens-Saprotrophs	Cosmopolitan	From *Pithomyces chartarum* leaves - Diverse plant families, but especially Poaceae.	https://nt.ars-grin.gov/fungaldatabases

Pleurostoma richardsiae	A	Calosphaeriales	External	Pathogen	Widespread	Wood streaking, canker; also a human pathogen.On *Olea europaea* (Oleaceae), *Vitis vinifera* (Vitaceae), *Prunus dulcis* (Rosaceae) and reports from diverse hosts.	https://nt.ars-grin.gov/fungaldatabases
Purpureocillium lilacinum	A	Hypocreales	Mycangia	Animal pathogen	Widespread	Nematicide and human pathogen	Song et al., 2016
Talaromyces amestolkiae	A	Eurotiales	Head, abdomen, mycangia	Animal pathogen	Cosmopolitan	Talaromyces contains species that are medically important. Emerging pathogen of agricultural crops.	Tsang et al., 2017; Yilmaz et al., 2014
Talaromyces minioluteus	A	Eurotiales	Mycangia, external	Plant pathogens-Saprotrophs	Cosmopolitan	Post harvest fruit rot.	Palou et al., 2013
Trichoderma atroviride	A	Hypocreales	Abdomen, external	Saprotrophs-Fungal antagonist	Widespread	On numerous hosts. Soil, wood, numerous other substrates.	https://nt.ars-grin.gov/fungaldatabases
Trichoderma harzianum	A	Hypocreales	External	Saprotrophs-Fungal antagonist	Cosmopolitan	Found on roots and other plant parts on numerous hosts;causing soft roots	Bissett et al., 2015; Han et al., 2017
Xenoacremonium falcatus	A	Hypocreales	External	Unassigned	Asia, Europe	*Castanea sativa*; other substrates	Aghyeva et al., 2017

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Table 2. List of most abundant fungi detected with HTS from *Xylosandrus crassiusculus*

SPECIES	PHYLUM	ORDER	BODY PART	FUNCTIONAL GUILD	OCCURANCE	REPORTED HOST/SUBSTRATE	REFERENCE
Ambrosiella roeperi	A	Microascales	Head, abdomen, mycangia	Plant pathogen	Widespread	Fungal symbiont of *X. crassiusculus*	Harrington et al., 2014
Arthrinium arundinis	A	Xylariales	Head, abdomen, mycangia	Saprotroph-Opportunistic	Cosmopolitan	Plurivorous. Living and dead plant parts, air, animals, soi	https://nt.ars-grin.gov/fungaldatabases
Aspergillus chevalieri	A	Eurotiales	Abdomen	Animal pathogen	Subtropical and tropical regions	Isolated from air, soil, and various kinds of organic debris	Domsch et al., 1980
Aulographina pinorum	A	Incertae sedis	Abdomen	Plant pathogen	Europe	On *Pinus* sp.	https://nt.ars-grin.gov/fungaldatabases
Beauveria pseudobassiana	A	Hypocreales	Abdomen, Mycangia	Animal pathogen	Cosmopolitan	Wide insect host range; can survive in diverse environments.	Imoulanet al., 2017
Cladosporium aggregatocicatricatum	A	Capnodiales	Abdomen	Endophyte-Saprotroph	New Zealand, Europe, North America	Plant material . Genus associated with Ambrosia beetles	Bensch et al., Kinuura 2002
Cladosporium aphidis	A	Mycosphaerellales	Abdomen	Saprotroph	Soth Africa	Dead aphids and honey dew produced by aphids	Bensch et al., 2012
Cladosporium pulvericola	A	Capnodiales	Mycangia	Saprotroph-Pathogen	Widespread	Air and soil	Bensch et al., 2018
Cladosporium sphaerospermum	A	Capnodiales	Head, abdomen, mycangia	Saprotroph	Cosmopolitan	Wide-host range; decaying Citrus leaves and branches in Italy; soil; decaying stem	Dugan et al. 2008; Zalaret al.,2007
Corynespora cassiicola	A	Pleosporales	Abdomen	Plant pathogen-Saprotroph	Comopolitan	Multiple genera on flowers, fruits, leaves, roots, and stems	https://nt.ars-grin.gov/fungaldatabases
Curvibasidium cygneicollum	B	Incetae sedis	Head	Unassigned	Europe, Asia	On *Picea abies*	Kaitera et al., 2019
Diaporthe amygdali	A	Diaporthales	Mycangia	Plant pathogen	Widespread	Canker, blight	https://nt.ars-grin.gov/fungaldatabases
Diplodia corticola	A	Dothideales	Head, abdomen	Plant pathogen	Southern Europe, North Africa, North America	Cankers, dieback various host	https://nt.ars-grin.gov/fungaldatabases

Epicoccum plurivorum	A	Incertae sedis	Head	Saprotroph-Opportunistic	Australia, Asia	Herbaceous plants, trees and shrubs	https://nt.ars-grin.gov/fungaldatabases
Fusarium incarnatum	A	Hypocreales	Head	Plant pathogen	Warm temperate and tropical regions	Causes numerous anthracnose diseases; wilt and stem rot, stipe rot, fruit rot	https://nt.ars-grin.gov/fungaldatabases
Fusarium sambucinum	A	Hypocreales	Head	Plant pathogen	Cosmopolitan	Canker, dieback, root rot, storage rot of potatoes	https://nt.ars-grin.gov/fungaldatabases
Fusarium solani	A	Hypocreales	Head	Plant pathogen	Cosmopolitan	Broad host range; associated with ambrosia beetles	Sharma and Marques, 2018
Geosmithia pallida	A	Hypocreales	abdomen, mycangia, external	Plant pathogen	Widespread	Associated with bark beetle Pseudopithyophthorus pubipennis; Hosts: Quercus spp. (Fagaceae), Prunus spp., Malus (Rosaceae);Causing Foamy bark canker, dieback, death	Kolarik et al., 2017
Geosmithia putterillii	A	Hypocreales	abdomen, head, mycangia	Plant pathogen	North America, New Zealand	Numerous hosts, associated with subcorticolous insects	Kolarik et al., 2004
Microcyclosporella mali	A	Mycosphaerellales	Abdomen	Plant pathogen-Saprotroph	Europe, North America	Fruit , Malus pumila.Biotrophic, necrotrophic or saprobic on various plant tissue	https://nt.ars-grin.gov/fungaldatabases
Nigrospora oryzae	A	Trichosphaeriales	Mycangia	Saprotroph-Plant pathogen	Cosmopolitan	Saprophyte and weak parasite; leaf spot of Rosemary, cob and stalk rot of maize	https://nt.ars-grin.gov/fungaldatabases
Penicillium brevicompactum	A	Eurotiales	Head, abdomen, mycangia	Plant pathogen	Cosmopolitan	Rots on numerous host	https://nt.ars-grin.gov/fungaldatabases
Penicillium sumatraense	A	Eurotiales	Head, abdomen, mycangia	Plant pathogen	Asia	Blue mold on diverse host	Liu and Lu 2018
Sarocladium strictum	A	Hypocreales	Head, abdomen, mycangia	Plant pathogen	Cosmopolitan	Broad host range, associated with ambrosia beetle Euwallacea fornicatus	Farr and Rossman, 2020; Li et al., 2016
Talaromyces amestolkiae	A	Eurotiales	Head, abdomen, mycangia	Animal pathogen	Cosmopolitan	Talaromyces contains species that are medically important. Emerging pathogen of agricultural crops.	Tsang et al., 2017; Yilmaz et al., 2014
Taphrina carpini	A	Taphrinales	Head	Plant pathogen	Asia, Europe	Witch's brooms, leaf deformation	Fonseca and Rodrigues 2011

Trichophaea woolhopeia	A	Pezizales	Mycangia	Ectomycorrhiza	Europe	On soil	Duran 2012
Trichothecium roseum	A	Hypocreales	Abdomen	Plant pathogen	Cosmopolitan	On a wide variety of organic substrates	https://nt.ars-grin.gov/fungaldatabases
Wickerhamomyces ciferrii	A	Saccharomycetales	Head, abdomen, mycangia	Saprotroph-Antagonist	Cosmopolitan	Soil	Wolff et al., 2013
Wickerhamomyces sydowiorum	A	Saccharomycetales	Head, abdomen, mycangia	Saprotroph	Africa	Insect-associated species	Carvajal Barriga 2014

With the amplification of ITS1 region in some case is not possible to arrive to species level. Only the 30 most abundant OTUS (operational taxonomic units) at species level are included on table 2

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3. *Xylosandrus compactus* (Eichhoff)

Xylosandrus compactus (black twig borer or shot-hole borer) is a highly polyphagous pest of woody plants that probably originates from Asia and has been introduced to other parts of the world, most probably with the trade of plants and wood. It is widely distributed in Africa, Asia and South America. It has been introduced in the Pacific Islands, New Zealand, Southeastern USA, and more recently in Europe in Italy and Southern France (EPPO Alert-list, Rabaglia et al., 2006, Wood, 1982; Chong et al., 2009). It was first found in Europe in 2011 (Garonna et al., 2012) in urban parks of the Campania region of Italy. Then, the species has been recorded in Italy's Campania, Tuscany and Liguria, and recently emerged in South-east France. The first report in Europe of *X. compactus* and associated ambrosia fungi in a natural environment has been recorded in September 2016, in the Italian National Park Circeo, Central Italy, in the Latium Region (Vannini et.al., 2017).

The adult females are dark brown to almost shiny black, 1.4-1.9 mm long and about two times longer than wide. The small, wingless males are reddish black and measuring 0.9–1.3 mm in length (Hara & Beardsley, 1979). *Xylosandrus compactus* is a species in which males are born from unfertilized eggs (0.3 -0.5 mm) and females from fertilized ones. After mating, which primarily occurs between siblings just after adult emergence, the male remains in the gallery while the female leaves the tunnel through the entry hole and colonizes branches of new hosts, boring an entry hole and a subsequent brood gallery (Hara & Beardsley, 1979; Greco & Wright, 2015). (CABI Factsheet).

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3.1. List of fungi associated to *Xylosandrus compactus*

Table 3 shows the fungal species isolated from the *X. compactus* specimens in the Circeo Park (Italy). *Table 4 shows the 30 most abundant OTUS (operational taxonomic units) at species level detected by HTS methodology.*

Table 3. List of fungi isolated from *Xylosandrus compactus*

SPECIES	PHYLUM	ORDER	BODY PART	FUNCTIONAL GUILD	OCCURANCE	REPORTED HOST/SUBSTRATE	REFERENCE
Acremonium camptosporum	A	Hypocreales	Abdomen, head, mycangia	Saprotrophs	Europe, Asia	dead plants or soil dwellers	Park, Thuong, Nguyen and Burm Lee, 2017
Acrodontium salmoneum	A	Pleosporales	Mycangia	Animal pathogen	Widespread	Wide-host range	Steiman et al., 1995
Alternaria alternata	A	Pleosporales	Abdomen, head, mycangia	Plant pathogens-Saprotrophs	Widespread	Wide-host range	Feng, Zheng, 2007
Ambrosiella xylebori	A	Microascales	Abdomen, head, mycangia	Symbiont-plant pathogen	Cosmopolitan	Obligate, mutualistic symbionts of ambrosia beetles	Mayers et al., 205
Aspergilllus spelaeus	A	Eurotiales	Abdomen, mycangia	Endophytes	Worldwide	soils and rhizospheres, indoor and cave environments	Hubka et al., 2017
Aspergillus flavus	A	Eurotiales	Abdomen	Plant pathogens-Saprotrophs	Cosmopolitan	Multitudinous substrates;secondary pathogen of some plants. Rots of fruit and food where it produces highly	Hubka et al., 2017
Aspergillus versicolor	A	Eurotiales	Head	Plant pathogens-Saprotrophs	Cosmopolitan	Numerous biological substrates.	Behnke-Borowczyk et al., 2019
Beauveria bassiana	A	Hypocreales	Abdomen	Animal pathogen	Cosmopolitan	Extremely wide host range: It can exist in diverse ecological environments including soil, plants and insects.	Imoulan et al., 2017
Beauveria pseudobassiana	A	Hypocreales	External	Animal pathogen	Cosmopolitan	Wide insect host range; can survive in diverse environments.	Imoulanet al., 2017

Cladosporium cladosporioides	A	Capnodiales	Abdomen, head, mycangia	Saprotrophs-hyperparasitic	Cosmopolitan	Multiple genera in multiple families; Leaves, seeds, inflorescences, often dead plant material; Causing leaf spot and blight, flower blight, scab, sooty mold.	Barkat et al., 2016
Cladosporium perangustum	A	Capnodiales	Abdomen, head, mycangia	Saprotrophs	Widespread	Numerous hosts, associated with plants, fungi, food	Ogórek et al., 2012; Bensch et al., 2012
Cladosporium psychrotolerans	A	Capnodiales	Head, abdomen, mycangia	Saprotrophs	Europe , North America , Dominican Republic.	Hypersaline water in the Mediterranean basin, indoor and outdoor environment, occasionally from plants	Bensch et al., 2012
Cladosporium ramontenellum	A	Capnodiales	Abdomen, head	Saprotrophs	South Africa, North America	Fruit and other plant material	K. Bensch et al., 2012
Cladosporium sphaerospermum.	A	Capnodiales	Head, abdomen	Saprotrophs	Cosmopolitan	Wide-host range; decaying Citrus leaves and branches in Italy; soil; decaying stem	Dugan et al. 2008; Zalaret al.,2007
Cladosporium uwebrauniana	A	Capnodiales	Head, mycangia	Unassigned	Europe	Indoor environment	Benschet al., 2012
Clonostachys byssicola	A	Hypocreales	Abdomen, head, mycangia	Saprotrophs	Cosmopolitan	Fungi, plants	Alvindia and Hirooka, 2011
Clonostachys rosea	A	Hypocreales	Abdomen, head, external	Plant pathogens-Saprotrophs	Cosmopolitan	Various plant parts both living and newly killed,associated with bark beetle galleries	Nygren et al., 2018
Clypeosphaeria phillyreae	A	Amphisphaeriales	Abdomen	Unassigned	Europe	Phillyrea latifolia	Reblova, 2017
Cytospora acaciae	A	Diaporthales	Abdomen, head	Plant pathogen	worldwide	Broad-host range; Ceratonia siliqua in Spain	N. Jiang et al., 2020
Fomes fomentarius	B	Polyporales	Head	Saprotrophs	Temperate northern hemisphere	Broad host range; on dead or living hardwoods	Hashemi and Mohammadi, 2016.
Fusarium solani	A	Hypocreales	Abdomen, head	Plant pathogen	Cosmopolitan	Broad host range; associated with ambrosia beetles	Sharma and Marques, 2018
Fusarium sporothichioides	A	Hypocreales	Head, mycangia, external	Plant pathogen	Temperate and tropical regions	Numerous hosts; found on roots, leaves, seeds, fruit causing root rot, leaf spot, dieback, etc	Arias et al., 2013; Taheri et al., 2017
Geosmithia flava	A	Hypocreales	Head, abdomen	Plant pathogen	Europe, North America (USA: CA). Eurpoe, Asia and	Numerous hosts; Beetle galleries, other plant substrates. In association with bark beetles	Kolarik et al., 2007

					Australia		
Geosmithia pallida	A	Hypocreales	Abdomen, head, mycangia	Plant pathogen	Widespread	Associated with bark beetle Pseudopithyophthorus pubipennis; Hosts: Quercus spp. (Fagaceae), Prunus spp., Malus (Rosaceae);Causing Foamy bark canker, dieback, death	Kolarik et al., 2017
Geosmithia sp. 21 NL-2015	A	Hypocreales	abdomen, head	Unassigned	California	Wide range of host plants found in association with different bark and ambrosia beetles	Kolarik *et al.*, 2017
Paraphoma fimeti	A	Pleosporales	Mycangia	Saprotrophs	Cosmopolitan	Soil, dead plant tissues; Herbaceous and woody plants; roots of Juniperus comunis	Boeremaet al., 2004; De Gruyteret al., 2010;Moslemiet al., 2017
Penicillium citrinum	A	Eurotiales	Head	Saprotrophs	Cosmopolitan	Soil, decaying vegetation, variety of organic substrates	Kozakiewwicz, 1992
Penicillium coccotrypicola	A	Eurotiales	Abdomen	Saprotrophs	Australia	Galleries of palm seed borer Coccotrypes carpophagus; Archontophoenix cunninghamiana (Arecaceae)	Crous et al., 2014
Penicillium glabrum	A	Eurotiales	External	Saprotrophs	Cosmopolitan	Numerous substrates; Numerous hosts; causing post-harvest fruit rot	Duduk et al., 2017
Penicillium glabrum	A	Eurotiales	Mycangia	Saprotrophs	Cosmopolitan	Numerous substrates; Numerous hosts; causing post-harvest fruit rot	Duduk et al., 2017
Penicillium multicolor	A	Eurotiales	Abdomen, head	Plant pathogens-Saprotrophs	Asia; Europe; USA	Leaves, seeds, coniferous and broadleaved sp.	Visagie et al., 2013
Penicillium pancosmium	A	Eurotiales	Abdomen	Plant pathogens-Saprotrophs	Worldwide	on hardwood log, Isolated from soil, old *Armillaria mellea* on a hardwood log, *Piptoporus* (on Betula sp), nut of *Juglans cinerea* (butternut) and porcupine dung.	Houbraken et al.,2015
Peniophora meridionalis	B	Russulales	External	Plant pathogen	Europe	Wood; decidious species; *Ceratonia siliqua*, *Erica* sp. *Eucalyptus* sp., *Q. Ilex*, *Q. Pyrenaica*, *Pistacia lentiscus*, *P. Halepensis*, *Arbutus unedo*, *Castanea* sp., *Viburnum tinus*	https://www.gbif.org/species/2552347
Pestalotiopsis biciliata	A	Xylariales	External	Plant pathogens-Saprotrophs	Cosmopolitan	Within woody species, both conifers and broadleaf trees are reported as hosts.causing a various range of symptoms including leaf	Morales-Rodríguez et al., 2018

						spots, leaf blight, fruits rot as well as post-harvest diseases.	
Peziza ostracoderma	A	Pezizales	Mycangia	Saprotrophs	North America and Europe	Peat mold	Lohr et al., 2017
Phanerochaete livescens	B	Polyporales	Mycangia	Saprotrophs	Widespread	Alnus glutinosa, A. incana, A. hirsuta, Quercus sp., Fagus sylvatica, Populus tremula, Corylus avellana, Acer platanoides, Padus avium	Volubev et al., 2015
Sarocladium strictum	A	Hypocreales	External	Plant pathogen	Cosmopolitan	Broad host range, associated with ambrosia beetle *Euwallacea fornicatus*	Farr and Rossman, 2020; Li et al., 2016
Simplicillium lamellicola	A	Hypocreales	Head	Plant-parasitic-Entomopathogen	Widespread	broad spectrum of hosts and substrates, such as insects, plants, rusts, nematodes and mushrooms	De-Ping et al., 2019
Sistotrema brinkmannii	B	Cantharellales	Abdomen	Saprotrophs	Widespread	Usually on wood, sometimes on plant debris and basidiomata	https://nt.ars-grin.gov/fungaldatabases
Talaromyces amestolkiae	A	Eurotiales	Head, abdomen, mycangium, ext	Animal pathogen	Cosmopolitan	Emerging pathogen of agricultural crops.	Tsang et al., 2017; Yilmaz et al., 2014
Talaromyces purpurogenus	A	Eurotiales	Head	Animal pathogen	Cosmopolitan	Emerging pathogen of agricultural crops	Tsang et al., 2017; Yilmaz et al., 2014
Torrubiella alba	A	Hypocreales	Head, mycangia	Animal pathogen	Cosmopolitan	Obligate symbiont with plants, animals and other fungal species	Johnson et al., 2008
Trichoderma hamatum	A	Hypocreales	Head, abdomen mycangium, ext	Saprotrophs-Fungal antagonist	Cosmopolitan	Found on roots and other plant parts on numerous hosts; causing soft roots	Bissett et al., 2015; Han et al., 2017
Umbelopsis westeae	M	Mucorales	Abdomen	Saprotroph	Australia	Different soil substrates	Wang et al., 2013
Ustilaginoidea virens	A	Incertae sedis	Abdomen	Plant pathogen	Worldwide in rice-growing regions	Substrate: Inflorescence/infructescense; Host: Oryza sativa, Zeamays, Brachiariabrizantha (Poaceae).	Kumari and Sharma, 2017; Fan et al., 2016
Xenoacremonium falcatus	A	Hypocreales	Head	Unassigned	Asia, Europe	*Castanea sativa*; other substrates	Aghyeva et al., 2017

Table 4. List of most abundant fungi detected with HTS from *Xylosandrus compactus*

SPECIES	PHYLUM	ORDER	BODY PART	FUNCTIONAL GUILD	OCCURRENCE	REPORTED HOST/SUBSTRATE	REFERENCE
***Alternaria alternata* complex**	A	Pleosporales	Mycangium	Saprotrophs, human allergens, and plant pathogens.	Widespread	Wide-host range	Feng, Zheng, 2007
Ambrosiella xyleborii	A	Microascales	Mycangium	mycangial symbiont of ambrosia beetle X. compactus	Widespread	X. compactus (Eichhoff)	Mayers et al., 2015; EPPO 2018;
Aureobasidium pullulans	A	Dothideales	Head, Abdomen	Saprotroph-Plant pathogen	Cosmopolitan	On leaf surfaces and other plant parts; a common contaminant. Saprobic; also causing post harvest fruit rot and coalescing red rotting spots on stems	https://nt.ars-grin.gov/fungaldatabases
Boeremia exigua	A	Pleosporales	Abdomen	Opportunistic parasite	Cosmopolitan	Leaves, stems, roots, tubers, pods.	Gilardi et al., 2017
Camptophora hylomeconis	A	Chaetothyriales	Head, Abdomen, Mycangium	Unassigned	Asia	Decaying leaves	Yang et al., 2018
Cladosporium austrohemisphaericum	A	Capnodiales	Head, Abdomen	Saprotroph	New Zealand, Africa, China, Spain	Plant material	Bensch et al., 2015
Cladosporium ramotenellum	A	Capnodiales	Head	Plant pathogen	Africa, North America	Post harvest fruit rot	Swett et al., 2016
Clonostachys rosea	A	Hypocreales	Head, Abdomen	Plant pathogens-Saprotrophs	Cosmopolitan	Various plant parts both living and newly killed,associated with bark beetle galleries	Nygren et al., 2018

Curvibasidium cygneicollum	B	Microbotryomycetes	Abdomen	Plant pathogens-Saprotrophs	Northern Europe	Various substrates including soil, sea sediment, wood etc. Found on Norway spruce	Kaitera et al., 2019
Epicoleosporium ramularioides	A	Mycosphaerellales	Head, Abdomen, Mycangium	Hyperparasite	Asia	Rust fungi on leaves; Coleosporium phellodendri (Coleosporiaceae) on Phellodendron amurense (Rutaceae)	Videira et al., 2017
Fusarium merismoides	A	Hypocreales	Head	Plant pathogen	Widespread	On diverse plants; Living leaves and stems; Causing stem canker, basal stem rot, root rot, stalk rot	Grafenhan et al., 2011
Fusarium solani	A	Hypocreales	Head	Plant pathogen	Cosmopolitan	Broad host range; associated with ambrosia beetles	Sharma and Marques, 2018
Geosmithia pallida	A	Hypocreales	Mycangium	Plant pathogen	Widespread including Europe	Associated with bark beetle Pseudopithyophthorus pubipennis; Hosts: Quercus spp. , Prunus sp., Malus sp.; Causing Foamy bark canker, dieback, death	Kolarik et al., 2017
Gnomoniopsis paraclavulata	A	Diaporthales	Head, Abdomen, Mycangium	Endophyte	North America	Quercus alba	Sogonov et al., 2008
Gnomoniopsis smithogilvyi	A	Diaporthales	Mycangium	Plant pathogen	Europe, Australia, New Zeland, Asia	Castanea (Fagaceae); Fruit, bark, leaves; Causing galls, canker and rotting	Dar et al., 2015
Inocybe putilla	B	Agaricales	Head	Saprotroph	Cosmopolitan	Wood decay	Zotti et al., 2001
Nigrospora sphaerica	A	Trichosphaeriales	Head	Endophyte-Plant pathogen	Cosmopolitan	Saprobic or weakly parasitic on a wide variety of plants.	https://nt.ars-grin.gov/fungaldatabases
Penicillium glabrum	A	Eurotiales	Head, Abdomen, Mycangium	Plant pathogen	Cosmopolitan	Post harvest fruit rot	Kozakiewicz 1992

Pithomyces chartarum	A	Pleosporales	Head	Saprotroph-Plant pathogen	Cosmopolitan	Diverse plant families, but especially Poaceae. May cause leaf spot and discoloration	Nasehi et al-. 2014
Pseudonectria foliicola	A	Hypocreales	Head, Abdomen	Plant pathogen	North America, New Zealand, Europe	Leaf spot on Buxus sp	Spetik et al., 2020
Sarocladium strictum	A	Hypocreales	Head, Abdomen, Mycangium	Plant pathogen	Cosmopolitan	Broad host range, associated with ambrosia beetle *Euwallacea fornicatus*	Farr and Rossman, 2020; Li et al., 2016
Stemphylium vesicarium	A	Pleosporales	Abdomen	Plant pathogen	Cosmopolitan	Wide host range; Leaves, roots, seeds causing leaf spots	Basallote et al., 1999
Tausonia pullulans	B	Cystofilobasidiales	Head	Unassigned	Widespread	On soils	Groenewald el at., 2018
Truncatella angustata	A	Amphisphaeriales	Abdomen	Endophyte and plant pathogen	Cosmopolitan	Multiple genera; on fruit, leaves, stems; Causing leaf spot and fruit rot	https://mycocosm.jgi.doe.gov/Truan1/Truan1.home.html
Tubakia dryina	A	Diaporthales	Head	Plant pathogen	Europe, North America, New Zealand	Leaf spot	Harrintong and Mc New 2018
Verticillium dahliae	A	Hypocreales	Abdomen	Plant pathogen	Cosmopolitan	Wilt, discoloration in multiple genera	https://nt.ars-grin.gov/fungaldatabases
Zymoseptoria verkleyi	A	Mycosphaerellales	Head, Abdomen, Mycangium	Unassigned	Europe	On Poa annua	Crous et al., 2012
Cladosporium halotolerans	A	Mycosphaerellales	Testa	Saprotroph	Widespread	Soil and air	Bensch et al., 2012
Biappendiculispora japonica	A	Pleosporales	Abdomen	Unassigned	Asia	Lignicolous freshwater fungi	Bao et al., 2019
Plectosphaerella cucumerina	A	Phyllachorales	Testa	Plant pathogen	Cosmopolitan	Causes disease of various plants	https://nt.ars-grin.gov/fungaldatabases

4. *Xylosandrus germanus (blandford)*

X. germanus is an Ambrosia beetle native to Southeast Asia but has now also settled in Europe and North America. In North America, *X. germanus* was first registered in New York in 1932 and is now established in 28 US states and three Canadian provinces. The first report of *X. germanus* in Europe occurred in Germany in 1951. The populations are now established in many parts of the European Union (it has been detected in 21 European countries and in Russia). In most of these countries *X. germanus* is considered a pest species and is expected to spread again to suitable new sites but may not be detected for many years due to its cryptic behavior. The main routes of diffusion are human-assisted movement by moving wood and infested wood products and by natural dispersion.

The known host spectrum of *X. germanus* currently includes over 200 species of trees and shrubs in 51 families, including trees that grow in woodlands, plantations, ornamental plants, nurseries, orchards, along with recently felled trunks, stored lumber and stumps. Thin-barked deciduous trees in ornamental nurseries are more easily attacked than conifers but we know that *X. germanus* is capable of attacking stored timber of both broadleaf and conifers. Although *X. germanus* shows an apparent preference for trees with thin bark, it does not seem to discriminate on the diameter of the host material; it was indifferently found on stumps, twigs, branches and trunks.

This small beetle, which is about 2mm long, attacks and creates small holes 1mm in diameter to form tunnels in the wood of apparently healthy plants, stressed, dying, or recently dying plants. The tunnels are dug by the females and include the tunnel entrance, brood chambers containing eggs and intermediate stages and secondary tunnels where the young develop; this structure adapts to all stages of life and the development processes of the insect. The larvae go through 3 stages and development from the egg to the adult stage takes about 30 days. The species is bivoltine and overwinters as an adult in the galleries of host plants which are often found at the base of the trunk, and which can contain dozens of beetles.

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4.1. List of fungi associated to *Xylosandrus germanus*

Table 5 shows the fungal species isolated from the *X. germanus* specimens in the Circeo Park (Italy). *Table 6 shows the 30 most abundant OTUS (operational taxonomic units) at species level detected by HTS methodology.*

Table 5. List of fungi isolated from *Xylosandrus germanus*

SPECIES	PHYLUM	ORDER	BODY PART	FUNCTIONAL GUILD	OCCURANCE	REPORTED HOST/SUBSTRATE	REFERENCE
Ambrosiella grosmanniae	A	Microascales	Mycangia	Symbiont/plant pathogen	Cosmopolitan	Obligate, mutualistic symbionts of ambrosia beetles	Contarini et al., 2020
Annulohypoxylon stygium	A	Xylariales	Abdomen	Plant pathogen	Warm-temperate to tropical regions	Canker on various woody host	Hsieh et al., 2005
Aposphaeria corallinolutea	A	Pleosporales	Abdomen	Plant pathogen	Europe	Wilting on Kerria japonica and Fraxinus excelsior	De Gruyter et al., 2013
Biscogniauxia mediterranea	A	Xylariares	Abdomen	Plant pathogen	Widespread	Charcoal disease on several host	Ju et al., 1998
Botrytis galanthina	A	Helotiales	Abdomen	Plant pathogen	Europe	Leaf spot various host	Harrison 1979
Byssochlamys spectabilis	A	Eurotiales	Testa, abdomen	Endophyte-Pathogen	Widespread	Variuos plant host and in food (heat-treated products)	Wu et al., 2018
Cladosporium austrohemisphaericum	A	Capnodiales	Abdomen	Endophyte	Australia, New Zealand, Africa	Fruit and other plant material	Bensch et al., 2015
Cladosporium pseudocladosporioides	A	Capnodiales	Abdomen	Endophyte-Antagonist	Widespread	Plants, fungi foods	Bensch et al., 2010

Cladosporium ramotenellum	A	Capnodiales	Head, abdomen, mycangium	Plant pathogen	Africa, North America	Post harvest fruit rot	Swett et al., 2016
Cladosporium sphaerospermum	A	Capnodiales	Abdomen	Saprotroph	Cosmopolitan	Wide-host range; decaying Citrus leaves and branches in Italy; soil; decaying stem	Dugan et al. 2008; Zalaret al.,2007
Diaporthe eres	A	Diaporthales	Abdomen	Plant pathogen-Endophyte-Saprotrophs	Cosmopolitan	Canker, fruit rot, leaf spot	Bastide et al., 2017
Diaporthe foeniculina	A	Diaporthales	Testa	Plant pathogen-Endophyte-Saprotrophs	Widespread	Shoot blight, leaf spot; potential for infection of a wide range of fruits as an opportunistic pathogen	Dissanayake et al., 2017
Hypoxylon crocopeplum	A	Xylariales	Testa, abdomen	Saprotroph	Widespread	Wood decay	Lee et al., 2000
Mollisia ligni	A	Helotiales	Testa	Saprotroph	North America and Europe	On wood , various host	Dennis 1986
Penicillium steckii	A	Eurotiales	Abdomen	Saprotroph	Cosmopolitan	Ubiquitous; in soil, decaying vegetation, and a variety of organic substrates	Kozakiewicz 1992
Peniophora quercina	B	Russulales	Abdomen	Saprotroph	Temperate northern hemisphere	Wood decay 23	Boddy and Rayner 1984
Pestalotiopsis pini	A	Xylariales	Mycangium	Plant pathogen	Europe	On Stone Pine	Silva et al., 2020
Hyphodontia setulosa	B	Hymenochaetales	Abdomen	Saprotroph	North America	Various host	Nakasone and Burdsall 1995
Pseudosydowia eucalypti	A	Dothideales	Mycangium	Plant pathogen	South Africa, Asia, Australia, Europe	Leaf spot, Eucalyptus spp	Crous et al., 2019
Quambalaria cyanescens	A	Incertae sedis	Mycangium	Pathogenicity uncertain	Australia, Europe	Associated with bark beetles in Europe	Crous et al., 2019

Querciphoma carteri	A	Pleosporales	Abdomen	Saprotroph-Pathogen	North America and Europe	Probably not responsible for dieback, but only an opportunistic pathogen	De Gruyter et al., 2013
Sarocladium kiliense	A	Incertae sedis	Abdomen	Plant pathogen	Asia, Europe, North America, South America	Fruit rot	Summerbell et al., 2011
Sarocladium strictum	A	Hypocreales	Testa, abdomen	Plant pathogen	Cosmopolitan	Broad host range, associated with ambrosia beetle Euwallacea fornicatus	Farr and Rossman, 2020; Li et al., 2016
Trichoderma atroviride	A	Hypocreales	Abdomen	Saprotrophs-Fungal antagonist	Widespread	On numerous hosts. Soil, wood, numerous other substrates	Bissett et al., 2015

Table 6. List of most abundant fungi detected with HTS from *Xylosandrus germanus*

SPECIES	PHYLUM	ORDER	BODY PART	FUNCTIONAL GUILD	OCCURANCE	REPORTED HOST/SUBSTRATE	REFERENCE
Ambrosiella grosmanniae	A	Microascales	Mycangia	Symbiont/plant pathogen	Cosmopolitan	Obligate, mutualistic symbionts of ambrosia beetles	Contarini et al., 2020
Aulographina pinorum	A	Incertae sedis	Abdomen	Plant pathogen	Europe	Pinus spp. Biotrophs,parasites on living leaves, stems and fruits	Firmino and Pereira 2021
Ciboria batschiana	A	Helotiales	Head, abdomen, mycangium	Pathotroph-Plant pathogen	nd	Castanea spp.	Tedersoo et al., 20
Cladosporium aggregatocicatricatum	A	Capnodiales	Abdomen	Endophyte-Saprotroph	New Zealand, Europe, North America	Plant material . Genus associated with Ambrosia beetles	Bensch et al., Kinuura 2002

Cladosporium austrohemisphaericum	A	Capnodiales	Head	Saprotroph	New Zealand, Africa, China, Spain	Plant material	Bensch et al., 2015
Clonostachys rosea	A	Hypocreales	Head	Pathogen	Cosmopolitan	Broad-host range; associated with bark beetle galleries	Farr and Rossman 2020; Kirschner 2001
Colletotrichum acutatum	A	Phyllachorales	Head, abdomen, mycangium	Plant pathogen	Cosmopolitan	Living leaves	Guerber & Correll 2001
Corynespora cassiicola	A	Pleosporales	Mycangia	Plant pathogen-Saprotroph	Cosmopolitan	Multiple genera. Flowers, fruit, leaves, roots, stems	Dixon et al.,2009
Cylindrium algarvense	A	Hypocreales	Mycangia	Endophyte-Plant pathogen	Europe	Eucalyptus spp.	Cheew. And Crous (2018)
Diplodia sapinea	A	Dothidales	Abdomen	Plant pathogen	Cosmopolitan	Numerous host, on stems, twigs and needles	Cannon and Kirk 2007
Epicoleosporium ramularioides	A	Capnodiales	Head, abdomen, mycangium	Hyperparasite	Asia (South Korea)	Coleosporiaceae , Rutaceae	Videira,et al., 2016
Filobasidium wieringae	B	Filobasidiales	Abdomen	Saprotroph	Europe	Fruit and other plant material	
Fusarium solani	A	Hypocrelaes	Abdomen	Plant pathogen	Cosmopolitan	Broad host range; associated with ambrosia beetles	Sharma and Marques 208
Geosmithia putterillii	A	Hypocreales	Abdomen, head, mycangia	Plant pathogen	North America, New Zealand	Numerous hosts, associated with subcorticolous insects	Kolarik et al., 2004
Gnomoniopsos smithogilvyi	A	Incertae sedis	Head, abdomen, mycangium	Endophyte-Plant pathogen	Europe, Australia,New Zealand, America, Asia	Castanea spp.	Cannon & Kirk 2007
Hohenbuehelia leightonii	B	Agaricales	Head	Saprotroph	Europe	Plant material	Kirk et al., 2008
Kalmusia variispora	A	Pleosporales	Head, abdomen, mycangium	Plant pathogen	Asia, Europe	Vitis vinifera, Quercus brantii, Erica canes	Ariyaw & Hyde 2014
Lazia echinophila	A	Helotiales	Head	Saprotroph	Europe	Involucre chesnut	Korf 1982

Microcyclosporella mali	A	Mycosphaerellales	Abdomen	Plant pathogen-saprotroph	Europe, North America	Fruit.Malus pumila	Schroers and Crous 2010
Penicillium corylophilum	A	Eurotiales	Head, abdomen, mycangium	Entomopathogen	Widespread	Numerous host	Direck 1901
Penicillium digitatum	A	Eurotielaes	Head	Saprotroph	Cosmopolitan	Numerous host	Palou 2014
Pseudoneoconiothyrium euonymi	A	Pleosporales	Head, abdomen, mycangium	Wood saprotroph	Ucraine	Fallen branches of Euonymus europeas	Crows & Akulov (2018)
Retroconis fusiformis	A	Incertae sedis	Head	nd	Cosmopolitan	nd	Hoog, and Batenburg-van der Vegte, 1989
Rhodotorula mucilaginosa	A	Sporidiobolales	Head	Saprotroph-Pathogen	Cosmopolitan	Water and organic debris	Yeeh 1999
Sporidiobolus roseus	B	Poridiobolales	Mycangia	Saprotroph-Animal pathogen	nd	Wide range of habitats, aquatic systems	Cannon & Kirk 2007
Trichothecium roseum	A	Incertae sedis	Abdomen	Plant pathogen	Cosmopolitan	On a wide variety of organic substrates	Hamid et al., 2014
Vishniacozyma carnescens	A	Tremellales	Head, abdomen, mycangium	Saprotroph	Cosmopolitan	Plant material, water	Li 2020
Vishniacozyma victoriae	B	Tremellales	Head	Saprotroph	Europe; South America	Broad-host range	Gramisci et al., 2018
Xanthoria parietina	A	Teloschisales	Head, abdomen, mycangium	Symbiotroph-Lichenized	nd	Numerous host	Cannon & Kirk 2007
Xerocomus subtomentosus	B	Boletales	Mycangia	Symbiont	Cosmopolitan	Coniferus and deciduous forest	Nilson and Persson 1977

CONCLUSION

Fungi typically live in highly diverse communities composed of multiple ecological guilds. "Plant-pathogens" have been the most abundant fungal-guild present on the isolated fungi from *Xylosandrus crassiusculus*, *X. compactus and X.germanus*. Of particular interest is the finding of several plant pathogens associated to different parts of the insect and having as hosts the family of Fagaceae or other tree species. For example: *Cryphonectria parasitica*, the causal agent of chestnut blight, isolated form *X. crassiusculus* or *Pestalopsis biciliata*, the causal agent leaf blotch symptoms on Eucalyptus. The genus *Fusarium* and the species complex *Fusarium solani* (FSSC) were isolated from both *Xylosandurs* species. *Fusarium solani* it is a symbiotic fungus cultivated in tunnels of host plants by the female pest, which is attracted to volatiles from *F. solani* (Egonyu, 2017). Furthermore, members of genus *Fusarium* have been reported in association with other ambrosia beetles, and they are often reported as pathogenic to the host tree and other woody crops (i.e., avocado) in Sicily (Gugliuzzo *et al*., 2020). Species belonging to the genus *Fusarium* have diverse ecological functions as they can act as saprophytes, endophytes and animal and plant pathogens. It is important to mention that the genus *Fusarium* includes important plant pathogens that affect both forest and agricultural species by producing different types of wall-degrading enzymes (e.g., cellulases, glucanases and glucosidases) and mycotoxins such as beauvericin and fumonisins (Bezos, 2018; Sharma, 2018). *Geosmithia pallida* is a species native to Europe (Lynch *et al.,* 2014) and one of the most diffused fungal species in Mediterranean maquis, closely associated with alien species. *G. pallida* appear to be more a no specific commensal. It was reported from other plant-insect interactions, such as *Castanea sativa* and the Cynipidae wasp *Dryocosmus kuriphilus* (Morales-Rodríguez *et al*., 2019), *Carya illinoinensis* and *Quercus laurifolia* with *Pseudopityophthorus minutissimus* (Huang *et al*., 2019) or associated with *X. compactus* at the National Park of Circeo (Vannini *et al.,* 2017). But it can also behave as a plant pathogen, for instance, *G. pallida* have been reported in the literature as a causal agent of foamy bark canker in *Quercus agrifolia* in Californiain association with *Pseudopityophthorus pubipennis* (Lynch *et al.,* 2014). According to the literature, *G. pallida* was accidentally introduced from Europe, like an alien pathogen of live oaks in the United States (Lynch *et al.,* 2014).

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The introduction of ambrosia beetle *Xylosandrus* and consequently fungal species, which represent a prevalent group of forest pathogens, as they are the major component of biodiversity in Europe and second-largest group of Eucaryotes right after insects. Many fungal species are considered as cryptogenic, which means they are most likely alien but with unknown origin, as they are poorly represented in alien species databases and unfortunately there is poor knowledge of their biogeography. For example, species as *Paraconiothyrium archidendri* reported on Asia, *Acremonium roseolum* on Asia and South America or *Geosmithia* sp. 21 on USA; which roles should be more investigated.

Symbiosis plays a critical role when the insects attempt to invade a new habitat. Understanding the ecological factors that influence the adaptation of an organism in a new environment and the uptake of new microorganisms are the key to explain the mechanism of biological invasions. Right one of the most complex examples of symbiosis is the one between ambrosia beetles and ambrosia fungi. Nevertheless, should be considered that different kind of fungal species are associated to different species of ambrosia beetles, and this is the reason why some of the fungal species associated to some beetles are not found in association with *X. compactus*/*crassiusculus*/*germanus* or vice versa, as there is the difference between fungal species that are associated to an exotic or native ambrosia beetle species. After the introduction of an exotic species such as *Xylosandus*, in a new environment, there is a series of biotic and abiotic forces that greatly influence the community of organisms in association with the insect. It is considered that forest habitat strongly influences the diversity of fungal species associated with the exotic ambrosia beetles. The absence of adaptation of exotic species could limit its establishment in a new environment. However, gaining microorganisms native to the invaded environment may support the exotic species to overcome these ecological barriers (Rassati et al., 2019).

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