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RESEARCH

The almond bark beetle mycobiome: fungal associates of *Scolytus amygdali* within southern Mediterranean almond orchards

Mariangela Benedetta Costanzo[1,2] · Peter H. W. Biedermann[2] · Giorgio Gusella[1] · Agatino Russo[1] · Giancarlo Polizzi[1] · Antonio Gugliuzzo[1]

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Abstract
Bark beetles are fundamental drivers of forest ecosystem dynamics. However, some species within this group have recently also emerged as significant pests in environments managed by humans, including nurseries, orchards, and urban areas. Specifically, certain *Scolytus* species pose potential economic and ecological threats to stone fruit cultivation across the Mediterranean basin. Although the role of the mycobiome in mediating bark beetle–host interactions has been well documented for many forest models, the symbiotic associations between beetles and fungi in cultivated Mediterranean agroecosystems remain poorly understood. This study provides the first comprehensive characterization of the fungal community associated with the almond bark beetle *Scolytus amygdali* in southern Mediterranean almond orchards. Integrating culture-dependent isolations with culture-independent approaches, i.e., high-throughput sequencing, allowed us to assess the beetle mycobiome across beetle adults, gallery systems, and associated necrotic wood lesions. Molecular characterization revealed *Geosmithia*, *Paecilomyces*, and *Quambalaria* to be the dominant taxa within the *S. amygdali* mycobiome. Their frequent occurrence suggests that they may be recurrent associates in the gallery environment, although their functional roles remain unclear. Furthermore, metabarcoding analyses provided additional novel insights into the beetle mycobiome, identifying *Candida* and *Yamadazyma* yeasts as potential core constituents of the gut microbiome. Future research should prioritize elucidating the role of these putative fungal symbionts for the beetles, particularly the pathogenic potential on almonds, and the functional efficiency of *S. amygdali* as a vector. Overall, our findings elucidate the complex taxonomic diversity of these associations. Moreover, obtained results provide a foundational ecological framework to better understand to what extent these associations can threaten host plants and to develop future sustainable management strategies in managed ecosystems.

Keywords Fungal symbiont · *Geosmithia* · *Paecilomyces* · Phytopathogenic fungi · *Quambalaria* · Wood boring insects

1 Introduction

Bark beetles (Coleoptera: Curculionidae: Scolytinae) are generally regarded as integral components of natural ecosystems, where they perform vital ecological functions by rejuvenating forests and promoting biodiversity (Raffa et al. 2008; Beudert et al. 2015; Biedermann et al. 2019). However, certain bark beetle species can pose a significant economic and ecological threat to cultivated trees (Zeiri et al. 2018; Apak 2021; Gugliuzzo et al. 2023a; Çelebi and Kaplan 2025; Costanzo et al. 2026). In particular, heavy attacks by bark beetles in the genus *Scolytus* on stone fruit trees in orchards, such as peaches, plums, apricots and almonds, have recently received increasing attention in the Mediterranean area, due to their link with shifting climate patterns and water stress events (Costanzo et al. 2026). Although the importance of the bark beetle mycobiome in bark beetle-host tree interactions is well recognised, certain *Scolytus*-fungus associations, particularly those involving species that infest cultivated trees in orchards, remain largely unexplored.

✉ Antonio Gugliuzzo
antonio.gugliuzzo@unict.it

[1] Department of Agriculture, Food and Environment (Di3A), University of Catania, Catania, Italy

[2] Chair of Forest Entomology and Protection, University of Freiburg, Stegen, Germany

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Almond [=*Prunus dulcis* (Mill.) D.A. Webb] is one of the major cultivated crops in the Mediterranean region, with a high economic and social importance (Bassi et al. 2024; Decroocq et al. 2025). Spain (369,700 t), Turkey (200,000 t), Morocco (163,616 t) and Italy (74,960 t) (FAOSTAT, 2024) are among the top almond-producing countries (in shell) in the Mediterranean area. In this region, almond cultivation can be affected by several wood fungal pathogens responsible for almond decline syndrome, trunk and branch cankers and dieback (Gusella et al. 2021a, 2023; Goura et al. 2022; Antón-Domínguez et al. 2023; Aloi et al. 2024). In addition, it can be threatened by *Scolytus amygdali* Geurin-Meneville, or almond bark beetle (ABB), considered one of the emerging bark beetle species native to the Mediterranean (Mifsud and Knizek 2009; Costanzo et al. 2026). Reported for the first time as a pest of fruit trees in 1921, it is a predominant bark beetle species throughout the world (Picard 1921; Russo 1931; Cherif and Trigui 1990; Kinawy et al. 1991; Mendel et al. 1997). Even though the orchard-inhabiting bark beetles' species are usually considered "secondary" pests because they mostly attack trees already weakened by biotic or abiotic factors (Brin and Bouget 2018; Zeiri et al. 2018), in some countries the ABB is considered a major pest, as reported for Tunisia (Cherif and Trigui 1990; Zeiri et al. 2015, 2018). This emergent bark beetle is primarily associated with woody species in the Rosaceae family, such as almond, peach and apricot (Bolu and Legalov 2008; Zeiri et al. 2014, 2015), with three to five generations per year, according to regional climatic differences (Costanzo et al. 2026).

Bark beetles maintain ubiquitous associations with a diverse range of fungi (Kirisits 2004). Beetle-fungus relationships can vary from beneficial to neutral to deleterious, depending on whether the fungus exhibits mutualistic, commensal or antagonistic interactions (Klepzig and Six 2004; Lieutier et al. 2009; Six and Wingfield 2011; Biedermann and Vega 2020). Additionally, some fungal associates can support the colonization of trees by bark beetles through their phytopathogenicity, being responsible for vascular wilt diseases, thus increasing the economic impact of the attacks (Hulcr and Dunn 2011; Six and Wingfield 2011; Hofstetter et al. 2015; Gusella et al. 2023). In the following, the terms "associated/associations" can refer to a statistical beetle-fungus co-occurrence even when a functional connection is still unknown. Associated fungi can also include the phoretic ones, which are only superficially attached to the beetle surface. Otherwise, when a proven, beneficial functional connection is already known, the terms "symbiont/symbiosis" or "mutualist/mutualism" are preferred (Vega and Biedermann 2020; Hulcr et al. 2025).

Though relatively poorly studied, common associates of *Scolytus* spp. worldwide include *Geosmithia* (Hypocreales) and Ophiostomatoid fungi (Ophiostomatales and Microascales) (Costanzo et al. 2026). This last group contains some phytopathogenic fungi vectored by different *Scolytus* spp. In particular, *S. chikisanii* Niisima, *S. japonicus* Chapuis, *S. multistriatus* Marsham and *S. scolytus* Fabricius are known as vectors of *Ophiostoma ulmi* (Buismann) Nannf and *O. novo-ulmi* Brasier (Ophiostomatales), the causal agents of Dutch Elm Disease (Webber 2000; Kirisits 2004; Menkis et al. 2016; Pepori et al. 2025). To date, the ecological role of most fungal associates of *Scolytus* spp. is still under-investigated. It has been suggested that *Geosmithia* fungi can use volatile chemicals to interact with bark beetles. As an example, volatiles of the symbiotic fungus *G. morbida* Kolařík, Freeland, Utley and Tisserat, the causal agent of Thousand Canker Disease (TCD) on black walnut, attract *Pityophthorus juglandis* Blackman and may synergize beetle aggregation (Blood et al. 2018).

It has been proven also that *Geosmithia* spp. can be involved in disease pathosystems and can interact with other fungi in the beetle galleries. *Geosmithia* spp. and *O. novo-ulmi* are both consistently associated with *S. multistriatus* adults, sharing the same habitat within the host plant and during each developmental stage. It has been experimentally demonstrated that *Geosmithia* spp. can affect the growth and pathogenicity of *O. novo-ulmi* suggesting a parasitic association between them (Pepori et al. 2018, 2025). On the other side, Ophiostomatoid fungi were proven to detoxify the tree host's defensive chemistry, helping beetles to overcome metabolite toxicity, and provide possible chemical attraction for the bark beetles (Zaman et al. 2023).

The mycobiome of *S. amygdali* remains largely unexplored. To date, only *Quambalaria cyanescens* (de Hoog & G.A. de Vries) Z.W. de Beer, Begerow & R. Bauer was isolated from living adults in Syria (Kolařík et al. 2006; Stodůlková et al. 2008), while *Aspergillus flavus* Link. and *Fusarium oxysporum* von Schlechtendal were recovered from dead adults in Turkey (Asma et al. 2017). In this study, we assessed the variability of the almond bark beetle fungal community in different almond orchards located in southern Italy, by using both culture-dependent and culture-independent approaches for the first time. In particular, the *S. amygdali* mycobiome composition was investigated by characterizing the fungal species isolated from both emerging beetles and gallery systems. The potential occurrence of fungal phytopathogens from necrotic wood lesions was also assessed. Finally, results from the culture-dependent approach were compared with those from metabarcoding.

2 Materials and methods

2.1 Collection and identification of beetles and wood samplings

Surveys were conducted on specialized almond orchards located in Noto (36.9314376–14.9278956) and Maletto (37.843428–14.834417) (Sicily, Italy) in summer 2023, and in Scicli (36.777455, 14.714054) and Pietraperzia (37.371816–14.107858) (Sicily, Italy) in summer 2024, soon after signs of bark beetle infestations were first noticed. Sections of infested almond branches and trunks (~ 30 cm in length) were randomly collected from almond plants (four plants per site) showing signs of bark beetle infestation, occurrence of gummosis exudates from entry holes in the bark, and symptoms of wilting and internal wood necrotic lesions (Fig. 1). Wood sections were placed inside labelled plastic boxes and brought to the laboratory of Plant Pathology at the Department of Agriculture, Food and Environment, University of Catania, Italy. Boxes were kept at 24 ± 2 °C and 60 ± 10% relative humidity and checked every day for beetle emergence. Adult female beetles spontaneously emerging from infested wood sections were collected using sterile forceps and individually placed in sterile 1.5 mL vials for both insect identification and fungal isolations via culture dependent methods or stored in pure ethanol at 4 °C for metabarcoding.

The morphological identification of the bark beetle individuals emerged from infested almond branch and trunk sections was based on the description of the most common *Scolytus* species occurring in the Mediterranean region available in Mifsud and Knizek (2009). Based on this, all the male and female individuals that emerged from branch and trunk sections of the infested almond trees were morphologically identified as *S. amygdali*. No other bark beetles from *Scolytus* or any other genus emerged from the infested woody material.

2.2 Culture dependent isolation and identification of fungal associates

To evaluate the mycobiome diversity of the almond bark beetle, fungi were isolated and analysed separately from adult insects, active galleries, and necrotic wood. This sampling approach was selected to comprehensively characterize the fungal community variability across different ecological niches associated with the beetle.

2.2.1 Fungal isolation from beetle adults and their galleries

Soon after their emergence, six beetle adults per plant were processed to assess their associated fungal community. Each beetle was crushed within the vial containing a sterile Phosphate-Buffered Saline (PBS) solution (Gugliuzzo et

Fig. 1 **A**) Adults of *Scolytus amygdali* and fungal mycelium in galleries under the bark of an almond branch section. **B**) Gum exudates from entry holes of *Scolytus amygdali* on almond branch in the field. **C**) Internal wood necrosis and discoloration around galleries and entry holes. **D**) Dying almond tree after bark beetle attacks and wilting

al. 2023b). The resulting mixture was serially diluted (1:10, 1:100; 1:1000) and 200 µL of each dilution were plated on Malt Extract Agar (MEA, Lickson, Vicary, Italy) amended with 100 mg L^{-1} Streptomycin sulfate (Sigma-Aldrich, St. Louis, MO, USA) (MEAS) to prevent bacterial growth. There were two plates for each individual/concentration combination. Plates were incubated in the dark at 25 ° ± 1 °C. After five days of incubation, fungal colonies were inspected and the dilution 1:100 was selected as the best for counting fungal colonies (CFUs).

The Incidence (%) (I) for each fungal genus/site was calculated according to the following formula: $I = (S_B/S_{TB}) \times 100$, where S_B represents the number of beetles from which a fungal genus was isolated and S_{TB} the total number of beetles. Moreover, the Relative Frequency (%) of isolation (RF) was assessed by using the following formula: $RF = (N_F/N_{FTOT}) \times 100$, where N_F is the number of emerged cultures for each fungal morphotype/beetle and N_{FTOT} is the total number of emerged cultures/beetle. The counting was repeated after 8 days of incubation to avoid missing the slow-growing fungal colonies.

When occurring within active beetle galleries, including eight active galleries per tree (32 active galleries per site), visible fungal mycelium was scraped with a sterile needle under stereoscopic microscope, plated to MEAS and incubated in the dark at 25 °C until fungal colonies were large enough to be observed. Representative isolates for fungal genera were used for monoconidial culture and stored in the collection of the laboratory of Plant Pathology at the Department of Agriculture, Food and Environment, University of Catania.

2.2.2 Fungal isolation from internal wood necrotic lesions

Once in the laboratory, a group of infested wood sections (eight per tree and 32 per site) were immediately processed for fungal isolations from necrotic lesions. In particular, four small subsections (from 0.2 to 0.3 cm^2) per infested wood section, totalling 126 subsections per site, of internal wood colonized tissues around entry holes and galleries, where the necrotic lesion was actively progressing, were cut and surface-sterilized for 60 s in 1.5% sodium hypochlorite solution, rinsed in Sterile Deionized Water (SDW) for 60 s, dried on sterile absorbent paper under a laminar hood, and placed on MEAS to prevent bacterial growth (Gusella et al. 2021b). Subsections were plated and then incubated at 25 ° ± 1 °C for 3–7 days until fungal colonies were large enough to be examined. The Relative Frequency (%) of isolation for each genus/site (RF) was calculated as follows: $RF = (N_S/N_{STOT}) \times 100$, where N_N is the number of cultured sub-sections from which a fungal morphotype emerged, and N_{STOT} is the total number of cultured sub-sections. Subsequently, colonies of interest were sub-cultured on MEAS plates to obtain pure and monoconidial cultures before DNA extraction and to be stored as previously described.

2.2.3 DNA extraction, amplification and sequencing

Six representative isolates for each morphotype, based on differences related to macroscopic features, were selected for genomic DNA extraction and molecular identification, for a total of 42 isolates. A portion of mycelium from 1-week-old monoconidial cultures was scraped off and proceeded following manufacturer's instructions of the Zymo-BIOMICS 96 DNA Kit (Zymo Research, Germany). PCR was performed with primers reported in Table 1. Each PCR reaction mixture had a final volume of 10 µl consisting of 2 µl Q5® Reaction Buffer Pack (New England Biolabs), 0.1 µl of each primer (10 µM), 0.1 µl of dNTPs (10 mM), 1 µl of DNA solution, 0.1 µl of Q5® High-Fidelity DNA Polymerase (New England Biolabs) and 6.6 µl of Nuclease-free Water. The PCR conditions were as follows: 95 °C for 3 min, followed by 30 cycles of 95 °C for 30 s, 52 °C (TUB2), or 55–62 °C (RPB2), or 56 °C (ITS, LSU and CaM), or 57 °C (TEF-1α) for 30 s, and 72 °C for 1 min. The final extension step was 72 °C for 10 min. PCR products were visualized on 1% agarose gels (100 V for 30 min) and purified following manufacturer's instructions of the PCR Purification Kit - Column Kit (Thüringen, Germany). Purified DNA amplicons were quantified by a Qubit™ 4 Fluorometer (Invitrogen) and Sanger sequencing was performed by StarSEQ (Mainz, Germany) with the same primers as in PCRs. Forward and reverse DNA sequences were assembled and manually corrected, when necessary, by using MEGA X11: Molecular Evolutionary Genetics Analysis (Tamura et al. 2021) and FinchTV Version 1.4.0 (Geospiza, Inc.; Seattle, WA, USA). Sequences were submitted and are available in GenBank (Tables 2, 3 and 4).

2.2.4 Phylogenetic analysis

Before constructing the phylogenetic trees, all the new sequences were blasted against the National Center for Biotechnology Information's (NCBI's) GenBank nucleotide database to determine the closest sequences for the most appropriate taxonomic arrangements. The reference sequences of closely related species were retrieved from the NCBI Batch Entrez databases and added to the alignments. A complete overview of all the used sequences, including isolates from beetle adults, active galleries and necrotic wood tissues of the present study, can be found in Tables 2, 3 and 4. *Rasamsonia emersonii* (Stock) Houbraken & Frisvad (CBS 393.64) and *R. byssochlamydoides* (Stock & Samson) Houbraken & Frisvad (CBS 413.71) were used

Table 1 Primers used for PCRs of isolated fungi

Genera	Gene	Primer name	Sequence	References
Geosmithia	ITS	ITS1F	CTTGGTCATT TAGAGGAAG TAA	Gardes and Bruns 1993
		ITS4	TCCTCCGCTTA TTGATATGC	White et al. 1990
	RPB2	fRPB2-5 F	GAYGAYMGW-GATCAYTTYGG	Liu et al. 1999
		fRPB2-7cR	CCCATRGCTT-GTYYRCCCAT	Liu et al. 1999
	TUB2	T10	ACGATAGGTTC ACCTCCAGAC	Glass and Donaldson 1995
		Bt2b	ACCCTCAGTG TAGTGACCCT TGGC	O'Donnell and Cigelnik 1997
	TEF-1α	EF1-728 F	CATCGAGAAGT TCGAGAAGG	Pepori et al. 2015
		EF1-986R	TACTTGAAGGA ACCCTTACC	Pepori et al. 2015
Quambalaria	ITS	ITS1F	CTTGGTCATT TAGAGGAAG TAA	Gardes and Bruns 1993
		ITS4	TCCTCCGCTTA TTGATATGC	White et al. 1990
	LSU	NL1	GCATATCAATA AGCGGAGGAA AAG	O'Donnell 1993
		NL4	GGTCCGTGTTT CAAGACGG	O'Donnell 1993
Paecilomyces	ITS	ITS1	TCCGTAGGTGA ACCTGCGG	White et al. 1990
		ITS4	TCCTCCGCTTA TTGATATGC	White et al. 1990
	TUB2	Bt2a	GGTAACCAAA TCGGTGCTGC TTTC	Glass and Donaldson 1995
		Bt2b	ACCCTCAGTG TAGTGACCCT TGGC	Glass and Donaldson 1995
	CaM	cmd5	CCGAGTACAA GGAGGCCTTC	Hong et al. 2005
		cmd6	CCGATAGAGGT CATAACGTGG	Hong et al. 2005

as outgrops for *Paecilomyces* spp., *Phragmotaenium derxii* (M. Takashima & Nakase) Q.M. Wang, Begerow, F.Y. Bai & Boekhout (CBS 110079) was used as outgroup for *Quambalaria* sp., and *Emericellopsis pallida* Beliakova (CBS 490.71) was used as outgroup for *Geosmithia* spp. to root the trees.

For the phylogenetic analysis, individual alignment was performed for each combination marker/fungal genus with MAFFT in Unipro UGENE with the default settings (Okonechnikov et al. 2012). After alignment and trimming, ITS, TUB2 and CaM for *Paecilomyces* spp., ITS and LSU for *Quambalaria* spp., and ITS, RPB2, TUB2 and TEF-1α for *Geosmithia* spp. were concatenated using SequenceMatrix version 1.7 (Vaidya et al. 2011). Maximum-Likelihood (ML) analyses of the multi-gene datasets were performed using IQ-Tree version 3 (Wong et al. 2025) with model selection carried out using ModelFinder and followed by inference (option -m TEST) (Kalyaanamoorthy et al. 2017). The edge-linked proportional partition model was applied (option -p partition-file) (Chernomor et al. 2016). UltraFast Bootstrap (UFBoot) (Hoang et al. 2018) and Shimodaira–Hasegawa-like approximate Likelihood Ratio Test (SH-aLRT) (Guindon et al. 2010) were performed with 1000 replicates to maximize load balance and efficiency (options -B 1000 and -alrt 1000). Gaps (-) and missing characters were treated as unknown characters. Branches were considered highly reliable when both SH-aLRT≥80% and UFBoot≥95% (Minh et al. 2020).

3 Culture-independent identification of fungal associates on beetle adults

3.1 Libraries and sample data preparation

While four sites were included in the overall study, metabarcoding analysis was performed exclusively on samples from three sites. This specific analysis was integrated into the study after samplings at the initial site (Noto) had already been completed, rendering those samples unavailable for molecular processing. As a consequence, only the sites Maletto, Scicli and Pietraperzia are considered here.

A total of 22 bark beetle individuals were collected from different trees and sites. In particular, eight adult beetles belonged to the site Maletto, six to the site Scicli and eight to the site Pietraperzia (see Table 5 for specific ID samples), previously stored in individual vials within pure ethanol, were processed for culture-independent fungal identification. Samples were first left for 24 h under a chemical hood for evaporation of all traces of ethanol. They were then treated with liquid nitrogen to facilitate and ensure the complete cell lysis, and then mechanically ground before proceeding with DNA extraction using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Germany) according to the manufacturer's instructions. The isolated DNA was stored at -20 °C. To ensure the reliability of the fungal community results, three negative controls and a self-designed fungal mock community were included. The fungal mock community consisted of standardized concentrations of each isolate, including one isolate of *Paecilomyces lecythidis* C. Ram, one of *Q. cyanescens*, and three isolates of *Geosmithia*.

The amplicon libraries were sequenced on an Illumina MISeq using 2 × 250 bp cycles with each marker. Both

Table 2 Isolates used for phylogenetic analyses of *Paecilomyces*

Species	Isolate number	Substrate/ host	Location	GenBank accession no.			References
				ITS	TUB2	CaM	
Peacilomyces brunneolus	[T]CBS 370.70 = IFO 7563	food	Japan	EU037050	EU037068	EU037033	Samson et al. 2009
P. clematidis	[T]CBS 148466 = MEND-F-0560	*Clematis* L. 'Snow Queen'	Czech Republic	MZ923760	MZ927740	MZ927738	Spetik et al. 2022
P. clematidis	MEND-F-0561	*Clematis* L. 'Snow Queen'	Czech Republic	MZ923761	MZ927741	MZ927739	Spetik et al. 2022
P. dactylethromorphus	[T]CBS 251.55 = ATCC 11971	acetic acid	Brazil	FJ389951	FJ390002	FJ389960	Samson et al. 2009
P. dactylethromorphus	CBS 323.34 = IMI 058411	unknown	Japan	FJ389947	FJ390005	FJ389962	Samson et al. 2009
P. divaricatus	[T]CBS 284.48 = JCM 12812	pectin	Mexico	FJ389931	FJ389992	FJ389953	Samson et al. 2009
P. divaricatus	CBS 110429 = ATCC 10121	mucilage bottle with library paste	Usa	FJ389932	FJ389991	FJ389954	Samson et al. 2009
P. formosus	[T]CBS 990.73B = ATCC 10865	Unknown	Japan	FJ389929	FJ389993	FJ389978	Samson et al. 2009
P. formosus	CBS 296.93 = No. B-7	Human bone marrow	Uzbekistan	FJ389928	FJ389994	FJ389961	Samson et al. 2009
P. fulvus	[T]CBS 132.33 = IMI 058421	bottled fruit	United Kingdom	FJ389939	FJ389988	FJ389957	Samson et al. 2009
P. fulvus	CBS 135.62	fruite juice	Switzerland	FJ389943	FJ389989	FJ389976	Samson et al. 2009
P. laguncullariae	[T]CBS 373.70	*Laguncularia racemosa*	Brazil	FJ389944	FJ389995	FJ389965	Houbraken et al. 2020
P. laguncullariae	CBS 696.95	Pasteurized strawberries	Netherlands	FJ389945	FJ389996	FJ389970	Samson et al. 2009
P. lecythidis	[T]CBS 372.70 = IMUR 2191	*Lecythis unsitata*	Brazil	FJ389926	FJ389990	FJ389964	Samson et al. 2009
P. lecythidis	CBS 118899 = CMW 18169	Wood utility pole (*Eucalyptus*)	South Africa	PP191150	PP197738	PP197769	Visagie et al. 2024
P. lecythidis	**MGA 54**	*Scolytus amygdali*, gallery/*Prunus amygdalus*	Italy	**PZ027805**	**PZ048595**	**PZ055299**	This study
P. lecythidis	**MGA 131**	Necrotic wood of *P. amygdalus*	Italy	**PZ027806**	**PZ048596**	**PZ055300**	This study
P. lecythidis	**MGA 143**	*S. amygdali*, gallery/ *P. amygdalus*	Italy	N/A	**PZ048600**	**PZ055301**	This study
P. lecythidis	**MCI 9**	*S. amygdali*, beetle/ *P. amygdalus*	Italy	**PZ027807**	**PZ048598**	**PZ055302**	This study
P. lecythidis	**MCI 28**	*S. amygdali*, beetle/ *P. amygdalus*	Italy	**PZ027808**	**PZ048597**	**PZ055304**	This study
P. lecythidis	**MCI 101**	*S. amygdali*, beetle/ *P. amygdalus*	Italy	**PZ027809**	**PZ048599**	**PZ055303**	This study
P. lignorum	[T]CBS 24309 = CMW 8180	Wood utility pole (*Eucalyptus*)	South Africa	PP191153	PP197741	PP197772	Visagie et al. 2024
P. lignorum	CMW 18182	Wood utility pole (*Eucalyptus*)	South Africa	PP191154	PP197742	PP197773	Visagie et al. 2024
P. maximus	[T]CBS 371.70	*Annona squamosa*	Brazil	FJ389920	FJ389982	FJ389963	Samson et al. 2009
P. maximus	[T]CBS 113247	Soil from pine-apple field	Thailand	FJ389921	FJ390009	FJ389980	Samson et al. 2009; Visagie et al. 2024
P. niveus	[T]CBS 100.11	unknown	unknown	FJ389934	FJ389999	FJ389956	Samson et al. 2009

Table 2 (continued)

Species	Isolate number	Substrate/ host	Location	GenBank accession no.			References
				ITS	TUB2	CaM	
P. niveus	CBS 133.37	Milk of cow	USA	FJ389935	FJ390000	FJ389958	Samson et al. 2009
P. paravariotii	[T]FRR 5287	Unknown	Unknown	N/A	OP985493	OP985494	Urquhart et al. 2018; Urquhart and Idnurm 2023
P. penicilliformis	[T]CBS 46003 = CCF 5755	air	USA	LR679769	LR679768	LR778299	Crous et al. 2020
P. penicilliformis	CCF 6350	peach-mango juice	USA	LR736038*	LR778163	LR778165	Crous et al. 2020
P. tabacinus	[T]CBS 41098 = CCF 5290	tobacco leaves	USA	LT548280	MN969434	LT548288	Houbraken et al. 2020
P. variotii	[T]CBS 102.74 = CECT 2803	Unknown source	France	EU037055	EU037073	EU037038	Houbraken et al. 2020
P. variotii	CBS 338.51	Fruit juice	Switzerland	FJ389930	FJ390007	FJ389955	Samson et al. 2009
P. zollerniae	[T]CBS 374.70 = JCM 12808	*Zollernia ilicifolia* and *Protium* heptaphyllum, "Pausanto"	Brazil	FJ389933	FJ390008	FJ389966	Samson et al. 2009
Rasamsonia byssochlamydoides	[T]CBS 413.71 = DTO 49D6	Dry soil under *Douglas* fir	USA	JF417476	JF417460	JF417512	Houbraken et al. 2012
R. emersonii	[T]CBS 393.64 = DTO 48I1	compost	Italy	JF417478	JF417463	JF417510	Houbraken et al. 2012
Thermoascus crustaceus	[T] CBS 181.67	*Parthenium argentatum*	USA	FJ389925	FJ389981	FJ389952	Samson et al. 2009

[T] holotype or ex-type isolates; N/A = not available; *ITS-LSU; Isolates numbers in boldface were collected in this study; Genbank accession no. in boldface were sequenced in this study

amplification and sequencing were performed by the Institute of Clinical Molecular Biology (IKMB), Kiel University. Amplification of the ITS2 hypervariable region of the fungal ITS2 gene was performed via one-step polymerase chain reaction (PCR) using barcoded primers. Primers 5.8 S-Fun ACTTTYRRCAAYGGATCWCT and Rev ITS4-Fun AGCCTCCGCTTATTGATATGCTTAART were used for the amplification of the ITS2 region (Taylor et al. 2016; Li et al. 2019). The reactions were performed using a mixture (25 µl) containing 0.15 µl Phusion Hot Start II Polymerase (2 U/µl) with 5 µl reaction buffer (Thermo Fisher Scientific), 10 mM dNTP mix, 10 µM of each primer, 13 µl H2O (Invitrogen, Thermo Fisher Scientific), and 4 µl of template DNA. Amplification steps consisted of the following: an initial denaturing step of 96 °C for 2 min, which was followed by 32 cycles of 94 °C for 30 s, 58 °C for 1 min, 72 °C for 2 min, and an elongation step for 72 °C for 10 min. PCR products were then purified and normalized with the SequalPrep Normalization Plate kit (Thermo Fisher Scientific) according to the manufacturer's guidelines. Final equimolar libraries were sequenced using the paired-end MiSeq reagent kit nano (2 × 250 bp chemistry) on the MiSeq platform (Illumina Inc.).

All statistical analyses and visualization of the sequence output were performed in RStudio (v. 2023.12.0.369) with R v. v. 4.3.2 (Posit team, 2023). From demultiplexed sequences, the absence of primers was confirmed while considering all the possible primer orientations with 'dada2', 'ShortRead' and 'Biostrings' packages (Morgan et al. 2009; Callahan et al. 2016; Pagès et al. 2019). Quality profiles of forward and reverse raw reads were visualized before chose filtering and trimming parameters to apply with dada2 pipeline v. 1.30.0 (Callahan et al. 2016). The function "filterAndTrim" was performed with the options maxEE = c (6, 10), trimLeft = c (0, 3) and truncLen = c (250, 168). The error rates for forward and reverse reads were estimated with function "learnErrors" and then the pooled core sample inference algorithm was applied. Forward and reverse reads were merged by the function "mergePairs". An Amplicon Sequence Variants (ASVs) table was obtained. Chimeras were identified and removed with the function "removeBimeraDenovo" and the expected distribution of sequence lengths for the amplified ITS region (variable from 250 bp to 403 bp) was assessed. As a sanity check, the number of reads that successfully completed each step of the pipeline was examined and no significant drop was associated with any single step (Table 5).

Table 3 Isolates used for phylogenetic analyses of *Quambalaria*

Species	Isolate number	Substrate/host	Location	GenBank accession no.		References
				ITS	LSU	
Quambalaria coyrecup	[T]WAC12947	*Corymbia calophylla*	Australia	DQ823431	DQ823444	Paap et al. 2008
Q. coyrecup	WAC12948	*C. calophylla*	Australia	DQ823433	DQ823446	Paap et al. 2008
Q. coyrecup	WAC12950	*Corymbia ficifolia*	Australia	DQ823429	DQ823447	Paap et al. 2008
Q. coyrecup	WAC12951	*C. ficifolia*	Australia	DQ823430	DQ823448	Paap et al. 2008
Q. cyanescens	[T]CBS 357.73 = CMW 5583	skin of man	Netherlands	DQ317622	DQ317615	de Beer et al. 2006
Q. cyanescens	CBS 876.73 = CMW 5584	*Eucalyptus pauciflora*	Australia	DQ317623	DQ317616	de Beer et al. 2006
Q. cyanescens	WAC12952	*C. calophylla*	Australia	DQ823419	DQ823440	Paap et al. 2008
Q. cyanescens	WAC129555	*C. calophylla*	Australia	DQ823421	DQ823441	Paap et al. 2008
Q. cyanescens	BRIP48396	*Corymbia citriodora*	Australia	EF444874	N/A	Pegg et al. 2008
Q. cyanescens	BRIP48403	*C. citriodora*	Australia	EF444876	N/A	Pegg et al. 2008
Q. cyanescens	CCTU 1684	*Vitis vinifera*	Iran	MN006031	N/A	Narmani and Arzanlou 2019
Q. cyanescens	CCTU 1738	*V. vinifera*	Iran	MN013769	N/A	Narmani and Arzanlou 2019
Q. cyanescens	**MGA 95**	Necrotic wood of *P. amygdalus*	Italy	**PZ027851**	**PZ027834**	This study
Q. cyanescens	**MGA 122**	*S. amygdali*, gallery/ *P. amygdalus*	Italy	**PZ027852**	**PZ027835**	This study
Q. cyanescens	**MGA 159**	*S. amygdali*, gallery/ *P. amygdalus*	Italy	**PZ027853**	**PZ027836**	This study
Q. cyanescens	**MGA 161**	*S. amygdali*, gallery/ *P. amygdalus*	Italy	**PZ027854**	**PZ027837**	This study
Q. cyanescens	**MGA 164**	Necrotic wood of *P. amygdalus*	Italy	**PZ027855**	**PZ027838**	This study
Q. cyanescens	**MGA 166**	Necrotic wood of *P. amygdalus*	Italy	**PZ027856**	**PZ027839**	This study
Q. cyanescens	**MGA 172**	*S. amygdali*, gallery/ *P. amygdalus*	Italy	**PZ027857**	**PZ027840**	This study
Q. cyanescens	**MGA 173**	*S. amygdali*, gallery/ *P. amygdalus*	Italy	**PZ027858**	N/A	This study
Q. cyanescens	**MCI 168**	*S. amygdali*, beetle/ *P. amygdalus*	Italy	**PZ027859**	**PZ027841**	This study
Q. cyanescens	**MCI 311**	*S. amygdali*, beetle/ *P. amygdalus*	Italy	**PZ027860**	**PZ027842**	This study
Q. cyanescens	**MCI 313**	*S. amygdali*, beetle/ *P. amygdalus*	Italy	**PZ027861**	**PZ027843**	This study
Q. cyanescens	**MCI 322**	*S. amygdali*, beetle/ *P. amygdalus*	Italy	**PZ027862**	**PZ027844**	This study
Q. cyanescens	**MCI 328**	*S. amygdali*, beetle/ *P. amygdalus*	Italy	**PZ027863**	**PZ027845**	This study
Q. cyanescens	**MCI 331**	*S. amygdali*, beetle/ *P. amygdalus*	Italy	**PZ027864**	**PZ027846**	This study
Q. eucalypti	[T]CBS 118844 = CMW 1101	*Eucalyptus grandis*	South Africa	DQ317625	DQ317618	de Beer et al. 2006
Q. eucalypti	[T]CBS 119680 = CMW 1678	*E. grandis* clone NH58	South Africa	DQ317626	DQ317619	de Beer et al. 2006
Q. eucalypti	BRIP48493	*E. grandis*	Australia	EF444826	N/A	Pegg et al. 2008
Q. eucalypti	CERC 8481	*E. grandis*	China	KY615014	KY615051	Chen et al. 2017
Q. pitereka	CMW 6707	*Corymbia maculata*	Australia	DQ317627	DQ317620	de Beer et al. 2006
Q. pitereka	[T]DAR 19773	*Corymbia eximia*	Australia	DQ823423	DQ823438	Paap et al. 2008
Q. pitereka	CBS 118828 = CMW 5318	*C. citriodora* subsp. *variegata*	Australia	DQ317628	DQ317621	de Beer et al. 2006
Q. pitereka	QP45	*C. citriodora* subsp. *variegata*	Australia	DQ823425	DQ823439	Paap et al. 2008
Q. pitereka	BRIP48317	*Corymbia henryi*	Australia	EF444854	N/A	Pegg et al. 2008
Q. simpsonii	[T]CBS 124772	*Eucalyptus tintinnans*	Australia	GQ303290	GQ303321	Cheewangkoon et al. 2009

Table 3 (continued)

Species	Isolate number	Substrate/host	Location	GenBank accession no.		References
				ITS	LSU	
Q. simpsonii	CERC 8507	*Eucalyptus urophylla* × *E. grandis*	China	KY615037	KY615058	Chen et al. 2017
Q. simpsonii	CERC 8534	*E. urophylla* × *E. grandis*	China	KY615045	KY615060	Chen et al. 2017
Jaminaea rosea	[T]MCA5214	*Plumeria* sp.	Florida	KR912071	KR912073	Kijpornyongpan and Aime 2017
Microstroma album	RB 2072	*Quercus robur*	Germany	DQ317624	AF352052	Begerow et al. 2001; de Beer et al. 2006
M. bacarum	[T]CBS 6526 = IGC4391	*Ribes nigrum*	United Kingdom	DQ317629	AF190002	de Beer et al. 2006; Fell et al. 2000
M. juglandis	CBS 287.63 = AFTOL-ID 1919	*Juglans regia*	Netherlands	DQ789988	DQ789987	Begerow et al. 1997; Matheny et al. 2006
M. phylloplanum	[T]CBS 8073 = IGC4246	*Banksia collina*	Australia	DQ317630	AF190004	de Beer et al. 2006; Fell et al. 2000
Phragmotaenium derxii	[T]CBS 110079 = JCM 10217	dead leaf of *Oryza sativa*	Thailand	AB045707	AB052823	Takashima and Nakase 2001
Pseudomicrostroma glucosiphilum	[T]MCA4718 = CBS 14053	Air	Germany	KR912070	KR912072	Kijpornyongpan and Aime 2017
Sympodiomycopsis kandeliae	[T]CBS 11676 = FIRDI 007	flowers of *Kandelia* candel	China	GQ465043	GU047881	Wei et al. 2011
S. paphiopedili	[T]CBS 7429	nectar of *Paphiopedilum primurinum*	Japan	DQ317631	AF190005	de Beer et al. 2006; Fell et al. 2000
Volvocisporium triumfetticola	[T]RB 2070	*Triumfetta rhomboidea*	India	DQ317637	AF352053	de Beer et al. 2006

[T] holotype or ex-type isolates; N/A = not available; Isolates numbers in boldface were collected in this study; Genbank accession no. in boldface were sequenced in this study

The dynamic version of the fungal UNITE ITS database (https://unite.ut.ee/repository.php) was used as reference to assign taxonomy to our ASVs by the function "assignTaxonomy" with the option tryRC = TRUE to match also the reverse complement of each ASV against the reference database. Taxa that could not be assigned to a known family or genus (i.e., labeled as "Incertae sedis" or NA) were categorized as "Undefined" in the corresponding taxonomic level. The taxonomic composition up to genus level of the mock community successfully confirmed sequencing of the contained taxa. The 'phyloseq' package (https://joey711.github.io/phyloseq/) was then used to import all the dada2 pipeline outputs into a phyloseq object. The taxonomic composition of negative controls was used to verify contaminations by using "decontam" pipeline with both the prevalence method and the more aggressive classification threshold = 0.5 (Davis et al. 2018). In both cases, no contaminants were identified in the three negative controls, suggesting no statistically significant presence of contaminant sequences. Negatives and mock controls were then excluded from the sample set. For the final analysis, 22 samples with an average of 132,973 reads (min. 1,586; max 12,747 reads) and 221 ASVs were included. Microbial composition of fungi was studied up to genus level.

3.2 Fungal diversity statistical analyses

The alpha diversity analysis was performed on data collapsed to genus level, after that sequence reads of all samples were rarefied to 3,000 reads/sample. Rarefaction removed 3 samples and 24 ASVs from the dataset. This threshold was selected after a comparative evaluation of multiple sampling depths to ensure an optimal balance between taxonomic resolution and the preservation of biological replicates. The α-diversity was estimated with metrics Observed richness (OR), Shannon's index, Simpson's and the inverse Simpson's indexes with the "estimate_richness" function in the 'phyloseq' package (Xia and Sun 2023). To test whether there was a difference in fungal diversity among sampling sites, Kruskal – Wallis test was performed for each α-diversity metric.

Table 4 Isolates used for phylogenetic analyses of *Geosmithia*

Species	Isolate Number	Substrate/tree host	Location of collection	GenBank accession no.				References
				ITS	Tub2	Tefl-α (Intron)	RPB2	
Geosmithia armandii	[T]CFCC 57330	*Tomicus armandii*, beetle/*Pinus armandii*	China	OR160282	OR801683	N/A	N/A	Liang et al. 2024
G. armandii	CFCC 57347	*T. armandii*, beetle/ *P. armandii*	China	OR160290	OR801691	N/A	N/A	Liang et al. 2024
G. bombycina	[T]SNM933 = CGMCC3.20578	*Cryphalus eriobotryae*, gallery/ *Eriobotrya japonica*	China	MZ519395	MZ514861	N/A	OL825678	Zhang et al. 2022
G. bombycina	SNM934	*C. eriobotryae*, gallery/ *E. japonica*	China	MZ519396	MZ514862	N/A	OL825679	Zhang et al. 2022
G. brevistipitata	[T]SNM1616 = CGMCC3.20627	*Phloeosinus* cf. *hopehi*, gallery/ *Cupressus funebris*	China	OK584392	OK632375	N/A	OL825675	Zhang et al. 2022
G. brevistipitata	SNM1611	*P.* cf. *hopehi*, gallery/ *C. funebris*	China	OK584394	OK632374	N/A	OL825676	Zhang et al. 2022
G. brunnea	[T]CBS 42633 = Hulcr 11903	*Hypothenemus dissimilis*, beetle/ *Quercus* sp.	USA	KY872743	KY872753	N/A	KY882268	Huang et al. 2017
G. brunnea	CBS 142634 = Hulcr 10838	*Xylosandrus compactus*, beetle/ *Liquidambar styraciflua*	USA	KY872741	KY872751	N/A	KY882266	Huang et al. 2017
G. capensis	[T]CMW 64002 = WR01	*Lanurgus jubatus*, beetle/ *Olea europaea* subsp. *africana*	South Africa	PQ032691	PQ048054	N/A	PQ045591	Aylward et al. 2024
G. capensis	CMW 59302 = 9_WWB002	*Lanurgus* sp. 3, beetle/ *Widdringtonia cedarbergensis*	South Africa	PQ032699	PQ048052	PQ045566	PQ045590	Aylward et al. 2024
G. carolliae	[T]URM 7929	*Carollia perspicillata*	Brazil	MH989506	MH989534	N/A	N/A	Crous et al. 2018
G. carolliae	URM 7930	*C. perspicillata*	Brazil	MH989507	MH989535	N/A	N/A	Crous et al. 2018
G. cnesini	[T]CCF 4292 = MK1820	*Cnesinus lecontei*, gallery/ *Croton draco*	Costa Rica	AM947671	N/A	N/A	N/A	Kolařík and Kirkendall 2010
G. cnesini	CCF 3753 = MK1802	*C. lecontei*, gallery/ *C. draco*	Costa Rica	AM947670	N/A	N/A	N/A	Kolařík and Kirkendall 2010
G. cupressina	[T]CBS 147103 = 304a	*Phloeosinus bicolor*, beetle/*Cupressus sempervirens*	Israel	PV272725	N/A	N/A	PV273307	Zhao et al. 2025
G. eupagioceri	[T]CCF 3754	*Eupagiocerus dentipes*, gallery/*Paullinia brenesii*	Costa Rica	AM947666	N/A	N/A	N/A	Kolařík and Kirkendall 2010
G. fagi	CCF 6235 = 23114 TB	*Taphrorychus bicolor*, gallery/*Fagus sylvatica*	Poland	LR812775	LR813119	LR813176	N/A	Strzałka et al. 2021
G. fagi	[T]CCF 6234 = 81516bTB	*T. bicolor*, beetle/*F. sylvatica*	Poland	LR812785	LR813129	LR813182	N/A	Strzałka et al. 2021
G. fassatiae	[T]CCF 3334 = AK14/93	*Quercus pubescens*	Czech Republic	AJ578482	N/A	N/A	N/A	Kolařík et al. 2005
G. fassatiae	U140	*Hylocurus hirtellus*, gallery system/*Salix* sp.	USA	HF546272	N/A	N/A	N/A	Kolařík et al. 2017
G. flava	[T]CBS 115346 = CCF 3333	*Xiphydria* sp., beetle /*Castanea sativa*	Czech Republic	PV272724	N/A	N/A	PV273306	Zhao et al. 2025
G. flava	CNR120	*Ulmus minor*	Czech Republic	KR229927	KP990611	KR135539	N/A	Pepori et al. 2015
G. funiculosa	[T]CBS 149063 = CNR48	Bark beetle, gallery/*U. minor*	Czech Republic	KR229897	KP990579	KR135506	N/A	Pepori et al. 2015; Crous et al. 2022

Table 4 (continued)

Species	Isolate Number	Substrate/tree host	Location of collection	GenBank accession no.				References
				ITS	Tub2	Tefl-α (Intron)	RPB2	
G. fusca	[T]SNM1578 = CGMCC3.20626	*Xylocis tortilicornis*, gallery/*Phyllanthus emblica*	China	OK584388	OK632370	N/A	OL825661	Zhang et al. 2022
G. fusca	SNM1577	*X. tortilicornis*, gallery/*P. emblica*	China	OK584387	OK632371	N/A	OL825662	Zhang et al. 2022
G. granulata	[T]SNM1015 = CGMCC3.20450	*Sinoxylon* cf. *cucumella*, beetle/ *Acacia pennata*	China	MZ519398	MZ514864	N/A	OL825667	Zhang et al. 2022
G. granulata	SNM1013	*S.* cf. *cucumella*, beetle/ *A. pennata*	China	MZ519397	MZ514863	N/A	OL825668	Zhang et al. 2022
G. langdonii	[T]CCF 3332 = AK 142/98	*S. intricatus*, beetle/*Q. robur*	Czech Republic	KF808297	HG799887	N/A	HG799928	Kolařík et al. 2005, 2017
G. langdonii	U61 = CCF4338	*Cryphalus pubescens*, gallery system/*S. serpervirens*	USA	HF546245	HG799881	N/A	HG799929	Kolařík et al. 2017
G. lavendula	[T]CBS 344.48 = ATCC 10463	Culture contaminant	USA	OQ429598	N/A	N/A	OQ453997	Hou et al. 2023
G. lavendula	CBS 121748 = DLS 1628	*Xylosandrus mutilatus*, beetle/ *Vitus rotundifolia*	USA	PV272728	N/A	N/A	N/A	Zhao et al. 2025
G. longistipitata	[T]CCF 4210 = RJ279m	*Polygraphus poligraphus*, gallery/ *Picea abies*	Poland	HE604154	LR813140	LR813191	N/A	Strzałka et al. 2021
G. longistipitata	RJ278m	*P. poligraphus*, gallery/ *P. abies*	Poland	HE604124	N/A	N/A	N/A	Strzałka et al. 2021
G. luteobrunnea	[T]SNM261 = CGMCC3.20252	*Scolytus jiulianshanensis*, gallery/*Ulmus* sp.	China	MW222399	MW592395	N/A	OL825669	Zhang et al. 2022
G. luteobrunnea	SNM226	*Acanthotomicus suncei*, gallery/ *L. styraciflua*	China	MW222404	MW592392	N/A	OL825670	Zhang et al. 2022
G. magnispora	[T]CBS 139790	*Pityogenes calcaratus*, *beetle*/*Pinus halepensis*	Israel	PV272718	N/A	N/A	N/A	Zhao et al. 2025
G. microcorthyli	[T]CCF 3861	*Microcorthylus* sp., gallery/*Cassia grandis*	Costa Rica	FM986798	FM986793	FN429956	FM986794	Kolařík and Kirkendall 2010
G. morbida	[T]CBS 124664 = CCF 3879	*Pityophthorus juglandis*, beetle/*Juglans nigra*	USA	FN434081	N/A	N/A	LR535706	Kolařík et al. 2011; Zhao et al. 2025
G. morbida	CBS 124663 = CCF 4010	*J. nigra*	USA	PV272721	N/A	N/A	N/A	Zhao et al. 2025
G. multisociorum	[T]CMW 40739 = NM93	*Cryphalini* sp.1, beetle/*Virgilia oroboides* subsp. *oroboides*	South Africa	KJ513226	PQ048047	N/A	N/A	Aylward et al. 2024
G. multisociorum	CMW 59300 = 15_WWB010	*Lanurgus* sp. 3, beetle/*W. cedarbergensis*	South Africa	PQ032712	PQ048051	PQ045563	PQ045589	Aylward et al. 2024
G. obscura	[T]CCF 3422 = MK 86	*S. intricatus*, gallery/*Q. robur*	Czech Republic	AJ784999	N/A	N/A	N/A	Kolařík et al. 2005
G. obscura	CBS 121749	*X. mutilatus*, beetle/ *V. rotundifolia*	USA	KT155620*	KT155332*	N/A	PV273302	Zhao et al. 2025; Stielow et al. 2015
G. omnicola	[T]CNR5	*U. minor*	Czech Republic	KR229867	KP990546	KR135473	N/A	Pepori et al. 2015
G. omnicola	CNR13	*Ulmus laevis*	Czech Republic	KR229872	KP990551	KR135478	N/A	Pepori et al. 2015
G. omnicola	CMW 64021 = 27_PyS	*Ctonoxylon* sp., beetle/*Podalyria calyptrata*	South Africa	PQ032715	PQ048042	PQ045570	PQ045605	Aylward et al. 2024
G. omnicola	**MCI 145**	*S. amygdali*, beetle/ *P. amygdalus*	Italy	**PZ028052**	**PZ055305**	**PZ210623**	**PZ179910**	This study
G. oroboidis	[T]CMW 40732 = NM105	*Scolytoplatypus fasciatus*, beetle/ *V. oroboides* subsp. *oroboides*	South Africa	KJ533336	N/A	N/A	N/A	Machingambi et al. 2014

Table 4 (continued)

Species	Isolate Number	Substrate/tree host	Location of collection	GenBank accession no.				References
				ITS	Tub2	Tefl-α (Intron)	RPB2	
G. oroboidis	CMW 40742 = NM006	*S. fasciatus*, beetle/ *V. oroboides* subsp. *oroboides*	South Africa	KJ533338	N/A	N/A	N/A	Machingambi et al. 2014
G. pallida	[T]CCF 3053 = NRRL2037	Cotton yarn	United Kingdom	AJ578486	HG799817	N/A	HG799908	Kolarík et al. 2004, 2017
G. pallida	SNM1165	*Sin.* cf. *cucumella*, gallery/*A. pennata*	China	MZ519393	MZ514859	N/A	OL825666	Zhang et al. 2022
G. pallida	CBS 101067	starch	Netherlands	PV272730	N/A	N/A	PV273309	Zhao et al. 2025
G. pazoutovae	[T]CCF 6233 = 24Wc14SI	*S. intricatus*, beetle/*Q. robur*	Poland	LR812796	LR813138	LR813188	N/A	Strzałka et al. 2021
G. pazoutovae	CCF 6231 = 47316bSI	*S. intricatus*, beetle/*Q. robur*	Poland	LR812790	LR813133	N/A	N/A	Strzałka et al. 2021
G. proliferans	[T]CBS 142636 = Hulcr 12334	*Phloeotribus frontalis*, beetle/*Acer negundo*	USA	KY872744	KY872754	N/A	KY882269	Huang et al. 2017
G. proliferans	CBS 142637 = Hulcr 12335	*P. frontalis*, beetle/*A. negundo*	USA	KY872745	KY872755	N/A	KY882270	Huang et al. 2017
G. pulverea	[T]SNM885 = CGM: CC3.20255	*Dinoderus* sp., gallery/ *Gnetum luofuense*	China	MW222410	MW592388	N/A	OL825656	Zhang et al. 2022
G. pulverea	SNM887	*Crossotarsus emancipates*, *gallery*/Unknown tree host	China	MW222412	MW592384	N/A	OL825660	Zhang et al. 2022
G. pulverea	SNM270	*A. suncei*, gallery/*Liquidambar formosana*	China	MW222398	MW592387	N/A	OL825659	Zhang et al. 2022
G. pulverea	**MGA 28**	Necrotic wood of *P. amygdalus*	Italy	**PZ028072**	**PZ094324**	**PZ235968**	**PZ094335**	This study
G. pulverea	**MGA 29**	Necrotic wood of *P. amygdalus*	Italy	**PZ028073**	**PZ094329**	**PZ235975**	**PZ094336**	This study
G. pulverea	**MGA 49**	Necrotic wood of *P. amygdalus*	Italy	**PZ028074**	**PZ094325**	**PZ251072**	**PZ094337**	This study
G. pulverea	**MGA 88**	Necrotic wood of *P. amygdalus*	Italy	**PZ028075**	**PZ094330**	**PZ251070**	**PZ094338**	This study
G. pulverea	**MGA 158**	*S. amygdali*, gallery/ *P. amygdalus*	Italy	**PZ028076**	**PZ094326**	**PZ251071**	**PZ094339**	This study
G. pulverea	**MCI 166**	*S. amygdali*, beetle/ *P. amygdalus*	Italy	**PZ028077**	**PZ094331**	**PZ235971**	**PZ094340**	This study
G. pulverea	**MCI 213**	*S. amygdali*, beetle/ *P. amygdalus*	Italy	**PZ028078**	**PZ094327**	N/A	**PZ094341**	This study
G. pulverea	**MCI 217**	*S. amygdali*, beetle/ *P. amygdalus*	Italy	**PZ028079**	**PZ094332**	**PZ235969**	**PZ094344**	This study
G. pulverea	**MCI 218**	*S. amygdali*, beetle/ *P. amygdalus*	Italy	**PZ028080**	**PZ094333**	**PZ235970**	**PZ094342**	This study
G. pulverea	**MCI 223**	*S. amygdali*, beetle/ *P. amygdalus*	Italy	N/A	N/A	**PZ235972**	**PZ094345**	This study
G. pulverea	**MCI 236**	*S. amygdali*, beetle/ *P. amygdalus*	Italy	**PZ028081**	**PZ094334**	**PZ235973**	**PZ094346**	This study
G. pulverea	**MCI 320**	*S. amygdali*, beetle/ *P. amygdalus*	Italy	**PZ028082**	**PZ094328**	**PZ235974**	**PZ094343**	This study
G. pumila	[T]SNM1653 = CGM: CC3.20630	*Scolytus semenovi*, gallery/ *Ulmus* sp.	China	OK584389	OK632366	N/A	OL825653	Zhang et al. 2022
G. pumila	SNM1657	*S. semenovi*, gallery/ *Ulmus* sp.	China	OK584390	OK632367	N/A	OL825654	Zhang et al. 2022
G. putterillii	[T]NRRL 2024 = CCF 3052	*Beilschmiedia tawa*	New Zealand	AF033384	HG799816	N/A	HG799907	Kolarík et al. 2004, 2017

Table 4 (continued)

Species	Isolate Number	Substrate/tree host	Location of collection	GenBank accession no.				References
				ITS	Tub2	Tefl-α (Intron)	RPB2	
G. putterillii	SNM402	*Phloeosinus* sp., gallery/Lauraceae	China	MW584874	MW592405	N/A	N/A	Zhang et al. 2022
G. radiata	[T]SNM279=CGMCC3.20253	*A. suncei*, gallery/ *L. formosana*	China	MW222397	MW592402	N/A	OL825672	Zhang et al. 2022
G. radiata	SNM884	*A. suncei*, gallery/ *L. formosana*	China	MW222406	MW592400	N/A	OL825673	Zhang et al. 2022
G. rufescens	[T]MK 1803=CCF 3752	*Cnesinus lecontei*, gallery/*Croton drago*	Costa Rica	AM947668	N/A	N/A	LR535708	Kolařík and Kirkendall 2010
G. stellenboschiana	[T]CMW 64012=WR28	*Hypothenemus* sp., beetle / *Searsia angustifolia*	South Africa	PQ032681	PQ048061	N/A	PQ045598	Aylward et al. 2024
G. stellenboschiana	CMW 64020=WR49	*Hypothenemus* sp., beetle/ *S. angustifolia*	South Africa	PQ032685	PQ048067	N/A	PQ045604	Aylward et al. 2024
G. subfulva	[T]SNM1304=CGMCC3.20579	*Ernoporus japonicus*, beetle/*Hibiscus tiliaceus*	China	OK584385	OK632368	N/A	OL825651	Zhang et al. 2022
G. subfulva	SNM1298	*E. japonicus*, beetle/*H. tiliaceus*	China	OK584386	OK632369	N/A	OL825652	Zhang et al. 2022
G. ulmacea	[T]CNR23	*U. minor*	Czech Republic	KR229881	KP990560	KR135487	N/A	Pepori et al. 2015
G. ulmacea	CNR24	*U. minor*	Czech Republic	-	KP990561	KR135488	N/A	Pepori et al. 2015
G. xerotolerans	[T]CBS 144969=FMR 17085	darkened wall of a house	Spain	LS998789	LS998791	N/A	N/A	Crous et al. 2018
G. xerotolerans	SNM1618	*P.* cf. *hopehi*, gallery/*C. funebris*	China	OK584391	OK632372	N/A	N/A	Zhang et al. 2022
G. xerotolerans	**MGA 50**	Necrotic wood of *P. amygdalus*	Italy	**PZ028053**	**PZ055306**	**PZ210622**	**PZ028053**	This study
Geosmithia sp. 1	MK1790 = CCF 4529	*Hypoborus ficus*, gallery system/ *Ficus carica*	France	AM421094	N/A	N/A	N/A	Kolařík et al. 2007
Geosmithia sp. 1	MK944	*H. ficus*, gallery system / *F. carica*	Slovenia	AM421090	N/A	N/A	N/A	Kolařík et al. 2007
Geosmithia sp. 4	MK1722 = CCF 4278	*Pteleobius vittatus*, gallery system/*U. laevis*	Czech Republic	AM181466	HG799813	N/A	HG799904	Kolařík et al. 2008, 2017
Geosmithia sp. 9	14KaFJD	*Cryphalus piceae*, beetle/*Abies alba*	Poland	KY568172	KY568478	KY568678	N/A	Jankowiak and Bilanski 2018
Geosmithia sp. 9	5KaFJD	*Pityophthorus pityographus*, beetle/*A.alba*	Poland	KY568181	KY568487	KY568686	N/A	Jankowiak and Bilanski 2018
Geosmithia sp. 11	CCF 3555	*S. intricatus*, gallery system/*Quercus* spp.	Hungary	AM181419	KF853931*	N/A	N/A	Kolařík et al. 2008; Hamelin et al. 2013
Geosmithia sp. 11	CCF 3556	*S. intricatus*, gallery system/*Quercus* spp.	Hungary	AM181418	N/A	N/A	N/A	Kolařík et al. 2008
Geosmithia sp. 12	CCF 3557	*Hylesinus orni*, gallery system /*Fraxinus* spp.	Hungary	AM181431	N/A	N/A	N/A	Kolařík et al. 2008
Geosmithia sp. 12	U164	*Hylesinus oregonus*, gallery system /*Fraxinus* sp.	USA	HF546229	N/A	N/A	N/A	Kolařík et al. 2017
Geosmithia sp. 16	RJ34m	*P. pityographus*, gallery system/*P. abies*	Poland	HE604147	HE604182	HE604207	HE604259	Kolařík and Jankowiak 2013
Geosmithia sp. 16	RJ08m = CCF 4201	*P. pityographus*, gallery system/*P. abies*	Poland	HE604146	HE604181	HE604206	HE604234	Kolařík and Jankowiak 2013
Geosmithia sp. 22	CCF 3645	*Phloetribus scarabeoides*, gallery system/*O. europaea*	Jordan	AM421061	KF853941*	N/A	N/A	Kolařík et al. 2007; Hamelin et al. 2013

Table 4 (continued)

Species	Isolate Number	Substrate/tree host	Location of collection	GenBank accession no.				References
				ITS	Tub2	Tefl-α (Intron)	RPB2	
Geosmithia sp. 22	CCF 3652	*P. scarabeoides*, gallery system/*O. europaea*	Croatia	AM421062	N/A	N/A	N/A	Kolařík et al. 2007
Geosmithia sp. 24	MB247	*P. calcaratus*, beetle/*Pinus brutia*	Israel	KP691921	KP691931	KP691941	N/A	Dori-Bachash et al. 2015
Geosmithia sp. 24	MB135	*Orthotomicus erosus*, gallery/*P. halepensis*	Israel	KP691925	KP691935	KP691945	N/A	Dori-Bachash et al. 2015
Geosmithia sp. 25	MK1832	*Cryphalus abietis*, gallery/*A. alba*	Czech Republic	HE604128	HE604186	HE604218	HE604250	Kolařík and Jankowiak 2013
Geosmithia sp. 25	MK1835 = CCF 4205	*C. piceae* + *P. pityographus*, gallery/*A. alba*	Czech Republic	HE604127	HE604187	HE604219	HE604253	Kolařík and Jankowiak 2013
Geosmithia sp. 26	MK1828 = CCF 4293	*P. pityographus*, gallery /*Pinus sylvestris*	Czech Republic	HE604112	LN907592	N/A	LN907603	Kolařík and Jankowiak 2013; Kolařík et al. 2017
Geosmithia sp. 26	RJ26 = CCF 4222	*Pityogenes bidentatus*, gallery/ *P. sylvestris*	Poland	HE604106	LN907594	N/A	LN907602	Kolařík and Jankowiak 2013; Kolařík et al. 2017
Geosmithia sp. 27	CCF 4206 = RJ0919	*P. bidentatus*, gallery/ *P. sylvestris*	Poland	HE794978	N/A	N/A	HG799893	Kolařík and Jankowiak 2013; Kolařík et al. 2017
Geosmithia sp. 27	U308b = CCF 4605	*Pityophthorus* sp., gallery/*Pinus ponderosa*	USA	HF546309	HG799827	N/A	HG799919	Kolařík and Jankowiak 2013; Kolařík et al. 2017
Geosmithia sp. 29	MK1827b = CCF 4221	*C. piceae* + *P. pityographus*, gallery/*A. alba*	Czech Republic	HE604125	HE604184	HE604233	HE604248	Kolařík and Jankowiak 2013
Geosmithia sp. 29	MK1809b = CCF 4199	*C. piceae* + *P. pityographus*, gallery/*A.alba*	Czech Republic	HE604126	N/A	HE604232	HE604243	Kolařík and Jankowiak 2013
Geosmithia sp. 30	MK1843	*Pityogenes quadridens*, gallery/*P. sylvestris*	Estonia	HE604149	HE604195	HE604222	HE604255	Kolařík and Jankowiak 2013
Geosmithia sp. 30	MK1801 = CCF 4288	*Ips cembrae*, gallery/*Larix decidua*	Czech Republic	HE604132	HE604193	HE604216	HE604242	Kolařík and Jankowiak 2013
Geosmithia sp. 31	MK1825	*P. pityographus*, beetle/ *P. sylvestris*	Czech Republic	HE604151	HE604174	HE604227	HE604246	Kolařík and Jankowiak 2013
Geosmithia sp. 31	RJ73k	*P. pityographus*, beetle/ *P. sylvestris*	Poland	HE604145	HE604177	HE604231	HE604263	Kolařík and Jankowiak 2013
Geosmithia sp. 32	CCF 3554 = MK538	*Phloeosinus thujae*, gallery system/ *Chamaecyparis pisifera*	Czech Republic	AM181426	HG799885	N/A	HG799926	Kolařík et al. 2008, 2017
Geosmithia sp. 32	U130 = CCF 5242	*Phloesinus sequoiae*, gallery system/*S. serpervirens*	USA	HF546265	HG799888	N/A	HG799925	Kolařík et al. 2008, 2017
Geosmithia sp. 33	U418 = CCF 4598	*Scolytus praeceps*, gallery system /*Abies concolor*	USA	HF546331	HG799831	N/A	HG799923	Kolařík et al. 2017
Geosmithia sp. 34	U417	*S. praeceps*, gallery system /*A. concolor*	USA	HF546330	HG799830	N/A	HG799922	Kolařík et al. 2017

Table 4 (continued)

Species	Isolate Number	Substrate/tree host	Location of collection	GenBank accession no.				References
				ITS	Tub2	Tefl-α (Intron)	RPB2	
Geosmithia sp. 34	U218 = CCF 4604	*Ips plastographus*, gallery system / *Calocedrus decurrens*	USA	HF546295	HG799826	N/A	HG799918	Kolařík et al. 2017
Geosmithia sp. 35	U196 = CCF 4322	*Pityophthorus* sp., gallery system /*Pseudotsuga menziesii*	USA	HF546231	HG799823	N/A	HG799914	Kolařík et al. 2017
Geosmithia sp. 36	U316 = CCF 4328	*Pityophthorus* sp., gallery system / *Pinus muricata*	USA	HF546236	HG799828	N/A	HG799920	Kolařík et al. 2017
Geosmithia sp. 37	U197	*P. menziesii* gallery system / *Pityophthorus* sp.	USA	HF546288	HG799824	N/A	HG799915	Kolařík et al. 2017
Geosmithia sp. 38	U79	*Pseudopityophthorus pubipennis*, gallery system/ *Notholithocarpus densiflorus*	USA	HF546346	N/A	N/A	N/A	Kolařík et al. 2017
Geosmithia sp. 38	U95 = CCF 5241	*P. pubipennis*, gallery system /*Quercus acrifolia*	USA	HF546251	N/A	N/A	N/A	Kolařík et al. 2017
Geosmithia sp. 39	U323	*Pityophthorus juglandis*, gallery system / *Juglans hindsii*	USA	HF546314	N/A	N/A	N/A	Kolařík et al. 2017
Geosmithia sp. 40	U216	*C. pubescens*, gallery system / *P. menziesii*	USA	HF546293	N/A	N/A	N/A	Kolařík et al. 2017
Geosmithia sp. 40	U307a = CCF 4194	*Pityophthorus* sp., gallery system / *P. muricata*/	USA	HF546220	N/A	N/A	N/A	Kolařík et al. 2017
Geosmithia sp. 41	U215	*Cossoninae* sp., gallery system / *Artemisia arborea*	USA	HF546292	HG799825	N/A	HG799917	Kolařík et al. 2017
Geosmithia sp. 41	U64	*Scobicia declivis*, gallery system / *Umbellularia californica*	USA	HF546342	HG799832	N/A	HG799930	Kolařík et al. 2017
Geosmithia sp. 42	U166	*Phloesinus canadensis*, gallery system / *Chamaecyparis* sp.	USA	HF546279	HG799821	N/A	HG799912	Kolařík et al. 2017
Geosmithia sp. 42	U185b = CCF 5251	*Scolytus rugulosus*, gallery system / *Prunus* sp.	USA	HF546285	HG799822	N/A	HG799913	Kolařík et al. 2017
Geosmithia sp. 43	U205 = CCF 4203	*Pityogenes knechteli*, *Pityophthorus* sp., gallery system/ *P. ponderosa*	USA	HF546223	N/A	N/A	HG799916	Kolařík et al. 2017
Geosmithia sp. 44	CCF 4332	*Pityophthorus* sp., gallery system / *Pinus sabiniana*	USA	HF546240	N/A	N/A	N/A	Kolařík et al. 2017
Geosmithia sp. 44	CCF 4333	*Pityophthorus* sp., gallery system / *Pinus sabiniana*	USA	N/A	LN907590	LN907598	N/A	Kolařík et al. 2017
Geosmithia sp. 45	Hulcr 17004	*Pityophthorus annectens*, beetle/*Pinus taeda*	USA	MH426752	N/A	N/A	N/A	Huang et al. 2019
Geosmithia sp. 45	Hulcr 17006	*P. annectens*, beetle/*P. taeda*	USA	MH426753	N/A	N/A	N/A	Huang et al. 2019
Geosmithia sp. 46	Hulcr 11575	*Pseudopityophthorus minutissimus*, beetle/ *Quercus laurifolia*	USA	MH426748	N/A	N/A	N/A	Huang et al. 2019
Geosmithia sp. 46	Hulcr 18077	*Hypothenemus eruditus*, beetle/ *J. nigra*	USA	MH426766	N/A	N/A	N/A	Huang et al. 2019
Geosmithia sp. 47	Hulcr 11904	*H. dissimilis*, beetle/ *Q. laurifolia*	USA	MH426749	N/A	N/A	N/A	Huang et al. 2019
Geosmithia sp. 47	Hulcr 19182	*H. dissimilis*, beetle/ *Carya illinoinensis*	USA	MH426789	N/A	N/A	N/A	Huang et al. 2019
Geosmithia sp. 48	Hulcr 19190	*Phloeosinus dentatus*, beetle/ *Juniperus virginiana*	USA	MH426796	N/A	N/A	N/A	Huang et al. 2019

Table 4 (continued)

Species	Isolate Number	Substrate/tree host	Location of collection	GenBank accession no.				References
				ITS	Tub2	Tef1-α (Intron)	RPB2	
***Geosmithia* sp. 49**	**MGA 58**	*S. amygdali*, gallery/*P. amygdalus*	Italy	**PZ191469**	**PZ161272**	N/A	**PZ161275**	This study
***Geosmithia* sp. 49**	**MCI 48**	*S. amygdali*, beetle/*P. amygdalus*	Italy	**PZ191470**	**PZ161273**	**PZ251073**	**PZ161276**	This study
***Geosmithia* sp. 49**	**MCI 57**	*S. amygdali*, beetle/*P. amygdalus*	Italy	**PZ191471**	**PZ161274**	**PZ251074**	**PZ161277**	This study
***Geosmithia* sp. 50**	**MGA 115**	Necrotic wood of *P. amygdalus*	Italy	N/A	**PZ179905**	**PZ253371**	**PZ161278**	This study
***Geosmithia* sp. 50**	**MGA 120**	*S. amygdali*, gallery/*P. amygdalus*	Italy	**PZ191472**	**PZ179906**	**PZ253372**	N/A	This study
***Geosmithia* sp. 50**	**MGA 121**	*S. amygdali*, gallery/*P. amygdalus*	Italy	**PZ191473**	N/A	**PZ253373**	**PZ161279**	This study
***Geosmithia* sp. 50**	**MCI 316**	*S. amygdali*, beetle/*P. amygdalus*	Italy	**PZ191474**	**PZ179908**	**PZ253374**	N/A	This study
***Geosmithia* sp. 50**	**MCI 317**	*S. amygdali*, beetle/*P. amygdalus*	Italy	**PZ191475**	**PZ179907**	**PZ253375**	N/A	This study
Emericellopsis pallida	[T]CBS 490.71	*Pityophthorus* sp.	Ukraine	NR_145052	KC987138	KC998998	KC999034	Grum-Grzhimaylo et al. 2013

[T]holotype or ex-type isolates; N/A = not available; *direct submission. Isolates numbers in boldface were collected in this study; Genbank accession no. in boldface were sequenced in this study

4 Results

4.1 Culture dependent isolation and identification of fungal associates

4.1.1 Isolation of fungal associates from adults, galleries and internal wood necrotic lesions

Based on culture morphology, fungal colonies derived from beetle bodies resembled three distinct genera, i.e., *Paecilomyces*, *Geosmithia* and *Quambalaria*. *Paecilomyces* and *Geosmithia* were isolated from all sampling sites, while *Quambalaria* occurred in all sites except Noto. Relative Frequencies (± SD) by genus and sampling sites are shown in Table 6. Specifically, *Paecilomyces* was consistently present in all sites, with the highest mean RF in Pietraperzia (80.26%) and the lowest in Noto (29.22%). The mean RF of the other consistently isolated fungal genus *Geosmithia* ranged from 68.74% in Noto to 5.28% in Pietraperzia. Morphotypes belonging to the *Quambalaria* genus were more prevalent in Pietraperzia (14.45%) and less in Scicli (10.90%) and Maletto (4.82%). All the previous three fungal genera were also collected from active beetle galleries in all sites, except *Quambalaria* which not occurred in Noto and Scicli.

Fungal colonies isolated from internal wood necrotic lesions belong to the same previously reported genera. In particular, *Paecilomyces* was consistently isolated from all sampling sites, showing the highest mean RF in Pietraperzia (87.63%) and the lowest in Scicli (22.54%), while intermediate frequencies were observed for Noto (40.65%) and Maletto (62.42%). *Geosmithia* occurred in all sampling sites except Pietraperzia, ranging from 25.42% in Noto to 34.37% and 52.01% in Maletto and Scicli, respectively. *Quambalaria* was not detected in Noto, with mean RF values ranging from 2.38% in Scicli to 8.33% and 9.55% in Maletto and Pietraperzia, respectively. All remaining fungal and/or bacterial colonies appeared only sporadically and were considered as contaminants.

4.1.2 Phylogenetic analysis

The combined multilocus phylogeny of *Paecilomyces* consisted of 37 sequences, comprising 1,640 total aligned characters (CaM: 1-602; ITS: 603–1,164; TUB2: 1,165–1,640). Among these, 577 were parsimony-informative (257 from CaM, 111 from ITS and 209 from TUB2), while 771 distinct patterns, 122 singleton sites, and 940 constant sites were identified. Two sites contain only gaps or ambiguous characters. ModelFinder indicated as best-fit substitution models TN + I+G4 for CaM, TPM2u + F+I+G4 for ITS and HKY + I+G4 for TUB2. The ML tree shows that all the

Table 5 Number of reads that successfully completed each step of the pipeline of metabarcoding

IDSample	Site	input[a]	filtered[b]	denoisedF[c]	denoisedR[d]	merged[e]	nonchim[f]
DL051	Maletto	8681	8425	8297	8184	8090	7252
DL052	Maletto	9812	9473	9369	9374	9275	8378
DL053	Maletto	6724	6539	6459	6395	6344	5900
DL054	Maletto	7341	7097	6962	6861	6758	6081
DL055	Maletto	4751	4629	4548	4336	4290	3479
DL056	Maletto	4509	4267	4120	4011	3906	2612
DL057	Maletto	6130	6031	5939	5789	5758	4830
DL058	Maletto	8072	7564	7370	7306	7142	6034
DL059	Scicli	13,794	13,464	13,392	13,155	13,114	11,500
DL060	Scicli	13,054	12,383	12,170	11,873	11,737	9596
DL061	Scicli	6613	6423	6371	6161	6130	5762
DL062	Scicli	2677	2509	2286	2248	2069	1586
DL063	Scicli	14,646	13,634	13,375	13,261	13,045	12,747
DL064	Scicli	5913	5769	5639	5538	5432	4133
DL065	Pietraperzia	4542	4336	4187	3963	3846	3275
DL066	Pietraperzia	4688	4450	4298	4218	4113	3128
DL067	Pietraperzia	12,066	11,004	10,574	10,483	10,138	9287
DL068	Pietraperzia	2946	2854	2776	2681	2657	2154
DL069	Pietraperzia	9525	8960	8861	8676	8590	8347
DL070	Pietraperzia	8248	7688	7449	7423	7234	6421
DL071	Pietraperzia	5982	5892	5816	5660	5599	5215
DL072	Pietraperzia	6230	5974	5809	5798	5648	5256
MOCK	-	12,874	11,187	10,947	10,868	10,652	5255
NC1*	-	214	199	1	1	0	0
NC2*	-	767	732	17	14	13	10
NC3*	-	70	66	62	63	62	28

[a] all the input raw reads; [b] reads after quality filtering and trimming; [c] reads after denoising of forward sequences; [d] reads after denoising of reverse sequences; [e] reads successfully merged; [f] reads after chimeras' removal; *Negative control samples

Table 6 Incidence, Relative Frequency (mean RF ± SD), and minimum and maximum values of the most abundant fungal genera isolated from beetle adults by the culture-dependent method among sampling sites

Site	*Paecilomyces*		*Geosmithia*		*Quambalaria*	
	Incidence % [a]	Mean RF (%) [b] ± SD; Min and Max (%)	Incidence % [a]	Mean RF (%) [b] ± SD; Min and Max (%)	Incidence % [a]	Mean RF (%) [b] ± SD; Min and Max (%)
Noto (SR)	100	29.22 ± 14.22; 10.53 and 54.79	100	68.74 ± 14.53; 39.83 and 88.97	0	-
Maletto (CT)	72.72	31.27 ± 44.22; 2.43 and 100	77.27	63.40 ± 41.62; 68.57 and 100	45.45	4.82 ± 8.73; 1.49 and 25.71
Scicli (RG)	100	70.30 ± 26.82; 27.87 and 100	66.66	16.94 ± 25.04; 6.25 and 68.28	83.33	10.90 ± 16.11; 3.85 and 48.08
Pietraperzia (EN)	94.11	80.26 ± 32.86; 10.43 and 100	11.76	5.28 ± 20.91; 3.49 and 86.36	52.94	14.45 ± 25.65; 2.56 and 89.57

[a] Incidence is given as percentage of samples with presence of particular fungal genera; [b] Mean percentages were calculated considering all replicates, including those where the fungus was absent (0%)

isolates sequenced in this study (MGA 54, MGA 131, MGA 143, MCI 9, MCI 28 and MCI 101) clustered in three separate sub-clade of an high-supported clade (99.8% SH-alrt, 100% UFBootstrap) with ex-type (CBS 372.70) and one reference strain (CBS 118899) of *P. lecythidis*, placed separately from the other species of *Paecilomyces* (Fig. 2).

For *Quambalaria*, the combined dataset consisted of 48 sequences and 1,258 total aligned characters (ITS: 1-677; LSU: 678–1,258). Parsimony-informative sites were 300 (250 from ITS, 50 from LSU). Additionally, 508 distinct patterns, 188 singleton sites and 770 constant sites were detected. ModelFinder selected TPM2 + F+G4 for ITS and TNe+G4 for LSU as best-fit substitution models. According to the phylogenetic tree, all used strains of *Quambalaria* appeared to form a monophylum (98.4% SH-alrt, 97% UFBootstrap), whereas new sequenced isolates of this

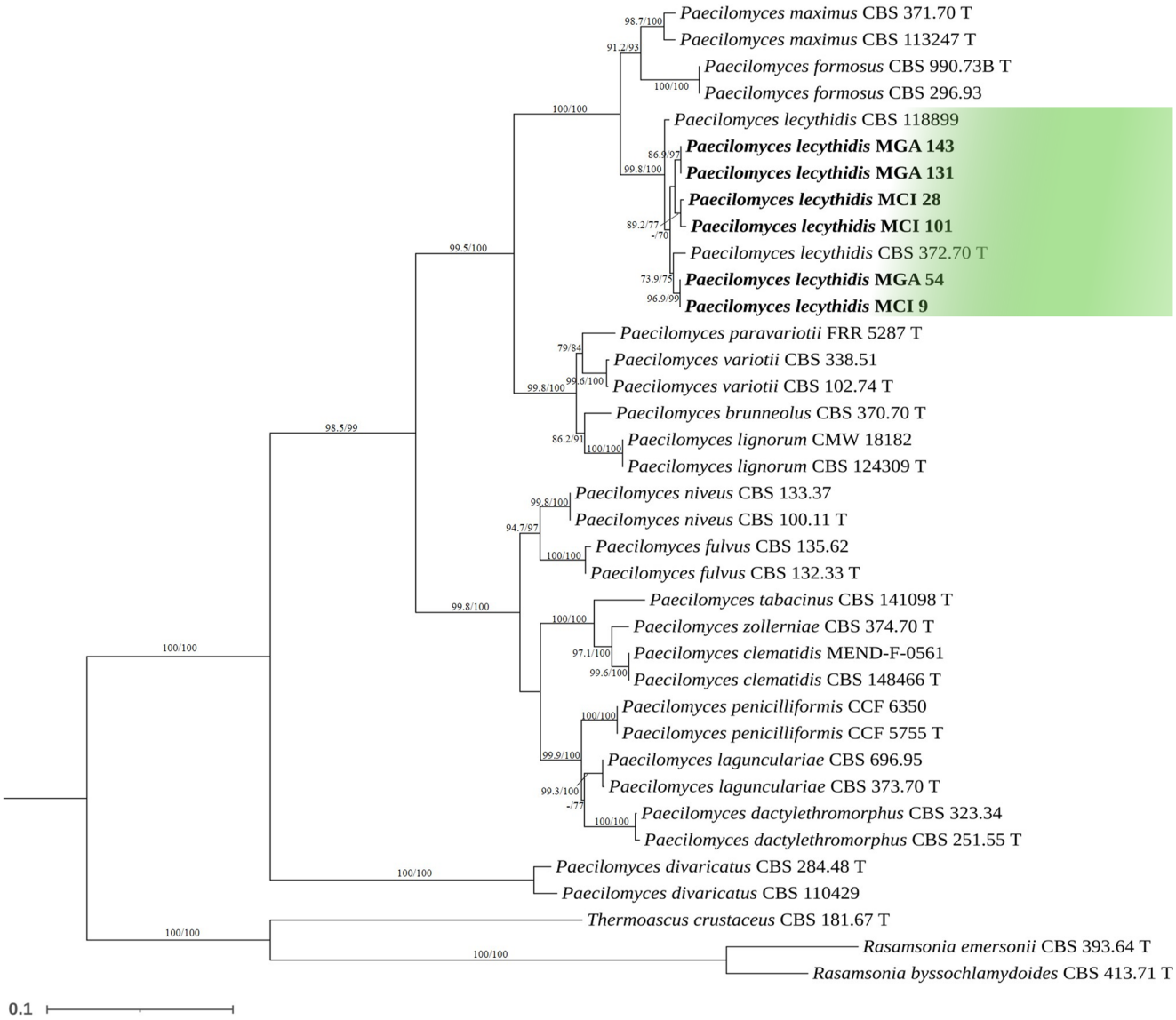

Fig. 2 Maximum Likelihood (ML) tree of *Paecilomyces* from the concatenated ITS, TUB2 and CaM sequence data. The sequences generated from this study are printed in bold. SH-aLRT and UFBoot support values above 70% are recorded at first and second position, respectively

study (MGA 95, MGA 122, MGA 159, MGA 161, MGA 164, MGA 166, MGA 172, MGA 173, MCI 311, MCI 313, MCI 322, MCI 328 and MCI 331) clustered in a clade with the ex-type and seven reference strains of *Q. cyanescens* (92.7% SH-alrt, 94% UFBootstrap) (Fig. 3).

Finally, the phylogenetic analysis of *Geosmithia* involved 150 sequences and 3,193 total aligned characters (ITS: 1-610; RPB2: 611–1,695; TEF-1α: 1,696–2,524; TUB2: 2,565–3,193). Among these, 1,285 were parsimony-informative (188 from ITS, 399 from RPB2, 332 from TEF-1α and 366 from TUB2), 1,785 were distinct patterns, 318 were singleton sites and 1,590 were constant sites. Two sites contain only gaps or ambiguous characters. The best-fit substitution models recommended were TIM2+F+I+G4 for ITS, TIM3+F+G4 for RPB2, HKY+F+G4 for TEF-1α and K3Pu+F+I+G4 for TUB2.

Isolates sequenced of the present study resided in five different clades, represented by *G. omnicola* Pepori, M. Kolarík, Bettini, Vettraino & Santini *G. xerotolerans* Rodr.-Andr., Cano & Stchigel, *G. pulverea* R. Chang & X. Zhang and two still undescribed species, i.e., *Geosmithia* sp. 49 and *Geosmithia* sp. 50. Specifically, our isolate MCI 145 clustered with the ex-type and two reference strains of *G. omnicola* whit a moderate support (100% SH-alrt, 88% UFBootstrap), whereas MGA 50 clustered with the ex-type and one reference strain of *G. xerotolerans* (78.4% SH-alrt, 100% UFBootstrap), and 12 isolates (MGA 28, MGA 29, MGA 49, MGA 88, MGA 158, MCI 166, MCI 213, MCI 217, MCI 218, MCI 223, MCI 236 and MCI 320) clustered with the ex-type and two reference strains of *G. pulverea* supported by high values (99.9% SH-alrt, 100% UFBootstrap). In addition, three (MGA 58, MCI 48 and MCI

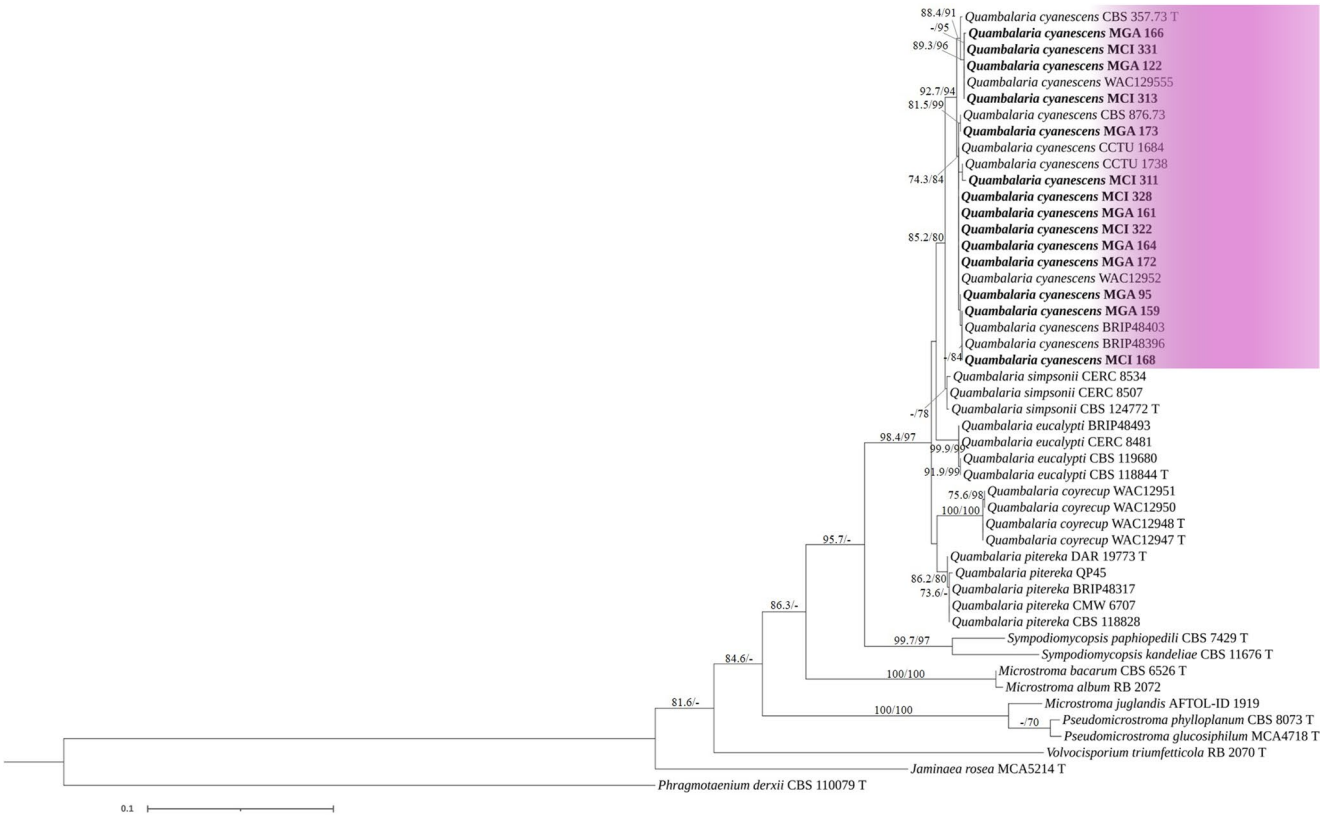

Fig. 3 Maximum Likelihood (ML) tree of *Quambalaria* from the concatenated ITS and LSU sequence data. The sequences generated from this study are printed in bold. SH-aLRT and UFBoot support values above 70% are recorded at first and second position, respectively

57) and five isolates (MGA 115, MGA 120, MGA 121, MCI 316 and MCI 317) clustered in two separate high-supported clade (84.1% SH-alrt, 100% UFBootstrap and 100% SH-alrt, 100% UFBootstrap, respectively) but not with ex-type strains and still undescribed species (Fig. 4).

Moreover, our results suggest *Geosmithia* sp. 49 and *Geosmithia* sp. 50 as undescribed and new *Geosmithia* species. The difference in number of isolates belonging to each species reflects that morphologically similar isolates belong to different species and vice versa, suggesting that molecular characterization is required to discriminate between species within the genus *Geosmithia*.

4.2 Culture-independent identification of fungal associates on beetle adults

4.2.1 Detected taxa in fungal dataset

Ascomycota and Basidiomycota phyla were detected across samples. In total, 67 families and 96 genera were recorded, excluding all the "undefined". Total fungal composition by families with different relative abundances (mean ± SD) is shown in Fig. 5. In particular, the most abundant families were Thermoascaceae and Quambalariaceae, whereas the most abundant detected genera were *Paecilomyces* with a relative abundance (mean % ± SD) of 40.74 ± 33.37 followed by *Quambalaria* (12.76 ± 17.02) and *Candida* (12.20 ± 25.53). Other species with a relative abundance exceeding 0.5% were *Yamadazyma* (8.03 ± 25.90), *Aspergillus* (7.36 ± 14.19), *Geosmithia* (2.87 ± 5.63), *Alternaria* (2.66 ± 6.42), *Stemphylium* (1.71 ± 5.80), *Aureobasidium* (1.59 ± 2.71), *Cladosporium* (1.37 ± 1.61), *Dioszegia* (0.61 ± 0.90) and *Calophoma* (0.54 ± 1.74). The relative abundance of the most abundant fungal genera was also assessed for each site. *Paecilomyces* was identified as the most dominant genus across all three sites, with mean relative abundances of 36.74 ± 21.82 (Maletto), 39.24 ± 45.00 (Scicli) and 45.86 ± 36.97 (Pietraperzia). This genus was followed by *Aspergillus* and *Quambalaria* in Maletto, *Candida* and *Quambalaria* in Scicli, and *Yamadazyma* and *Quambalaria* in Pietraperzia (Table 7; Fig. 6).

4.2.2 Alpha diversity

Rarefaction analysis indicated that the curves approached a plateau at the selected threshold of 3000 reads/samples (Figure S1). This indicates that this depth was sufficient to

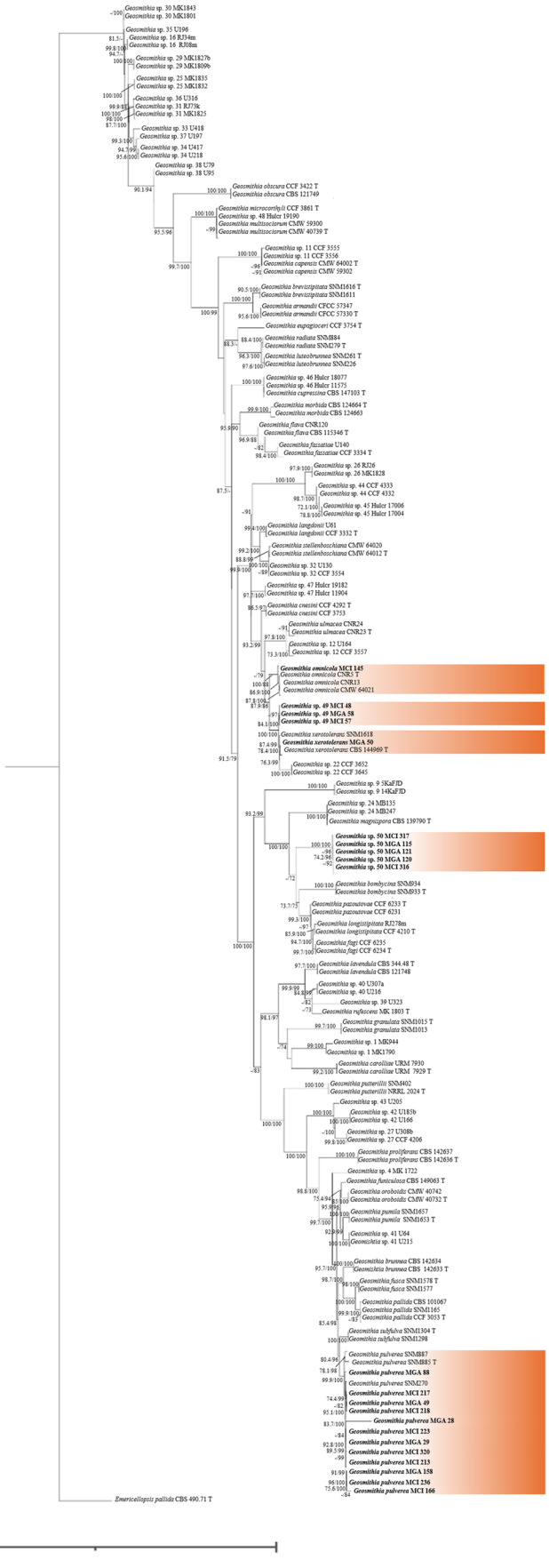

Fig. 4 Maximum Likelihood (ML) tree of *Geosmithia* from the concatenated ITS, RPB2, TEF-1α and Tub2 sequence data. The sequences generated from this study are printed in bold. SH-aLRT and UFBoot support values above 70% are recorded at first and second position, respectively

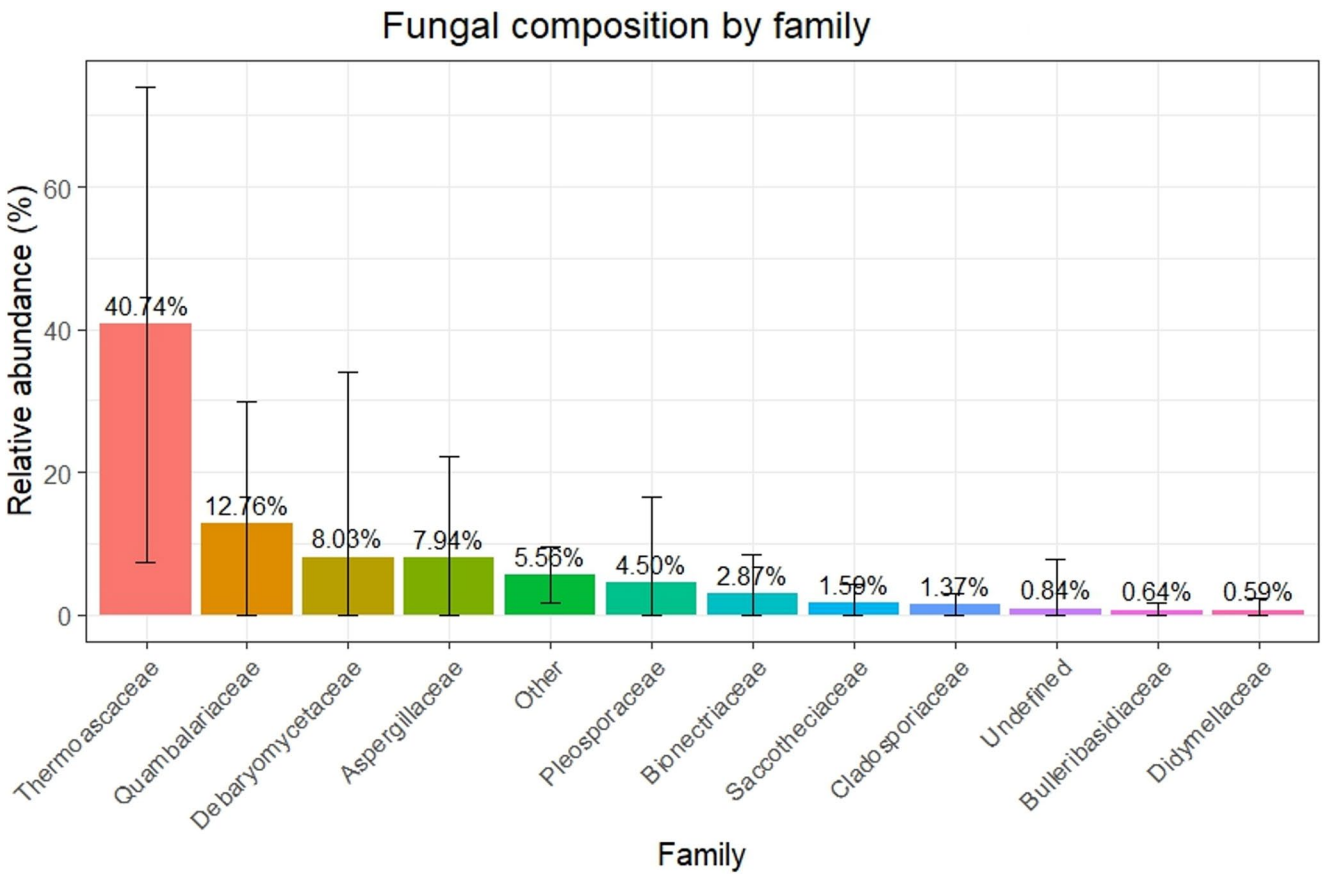

Fig. 5 Total fungal composition by families with different relative abundances ± SD detected by the culture-independent method. Only families with a total relative abundance > 0.5% are reported (all the others are combined in "Other")

Table 7 Relative Abundance (RA) (mean ± SD) of the most abundant fungal genera among sampling sites detected by the culture-independent method

Genus	Mean RA ± SD		
	Maletto	Scicli	Pietraperzia
Paecilomyces	36.74 ± 21.82	39.24 ± 45.00	45.86 ± 36.97
Quambalaria	19.08 ± 17.70	5.55 ± 9.99	11.85 ± 19.83
Candida	5.37 ± 3.19	37.59 ± 40.45	0.00 ± 0.00
Yamadazyma	0.03 ± 0.08	0.00 ± 0.00	22.05 ± 40.74
Aspergillus	20.14 ± 17.62	0.07 ± 0.10	0.05 ± 0.11
Geosmithia	7.65 ± 7.33	0.33 ± 0.80	0.00 ± 0.00
Alternaria	0.79 ± 1.22	3.18 ± 4.66	4.13 ± 9.99
Stemphylium	0.00 ± 0.00	2.22 ± 5.43	3.05 ± 8.62
Aureobasidium	3.04 ± 3.94	0.41 ± 0.42	1.03 ± 1.55
Cladosporium	1.42 ± 0.67	2.01 ± 2.85	0.82 ± 0.92
Dioszegia	0.24 ± 0.35	0.48 ± 0.90	1.08 ± 1.14
Calophoma	0.06 ± 0.10	0.04 ± 0.09	1.40 ± 2.78

capture the majority of the taxonomic diversity across all investigated sites while maintaining statistical power across replicates. According to tested alpha diversity's metrics, there was no significant difference in the observed richness at genus level (OR: $\chi^2 = 0.568$, $df = 2$, $p = 0.752$). Shannon, Simpson and 1/Simpson indexes detected no significant differences among sites, suggesting limited variation in genera richness and relative abundance (evenness) across all sites (Shannon: $\chi^2 = 3.825$, $df = 2$, $p = 0.147$; Simpson and inverse Simpson: $\chi^2 = 4.096$, $df = 2$, $p = 0.129$) (Fig. 7).

5 Discussion

This study provides the first comprehensive overview of the fungal communities associated with the bark beetle *S. amygdali* in almond orchards located in a southern Mediterranean area. The composition and diversity of *S. amygdali* fungal associates is here unveiled by using both culture-dependent methods and high-throughput sequencing technologies. Our results indicate that the mycobiome of this bark beetle is dominated by fungi belonging to the Ascomycota phylum. This finding corroborates previous studies on bark beetle–fungi associations (Zhang et al. 2022; Pineda-Mendoza et al. 2024; Costanzo et al. 2026). Interestingly, outcomes of the α-diversity analyses showed no significant difference both in terms of richness and evenness of the *S. amygdali* fungal community at site level. While metabarcoding outcomes provided a broad community profile, the culture dependent

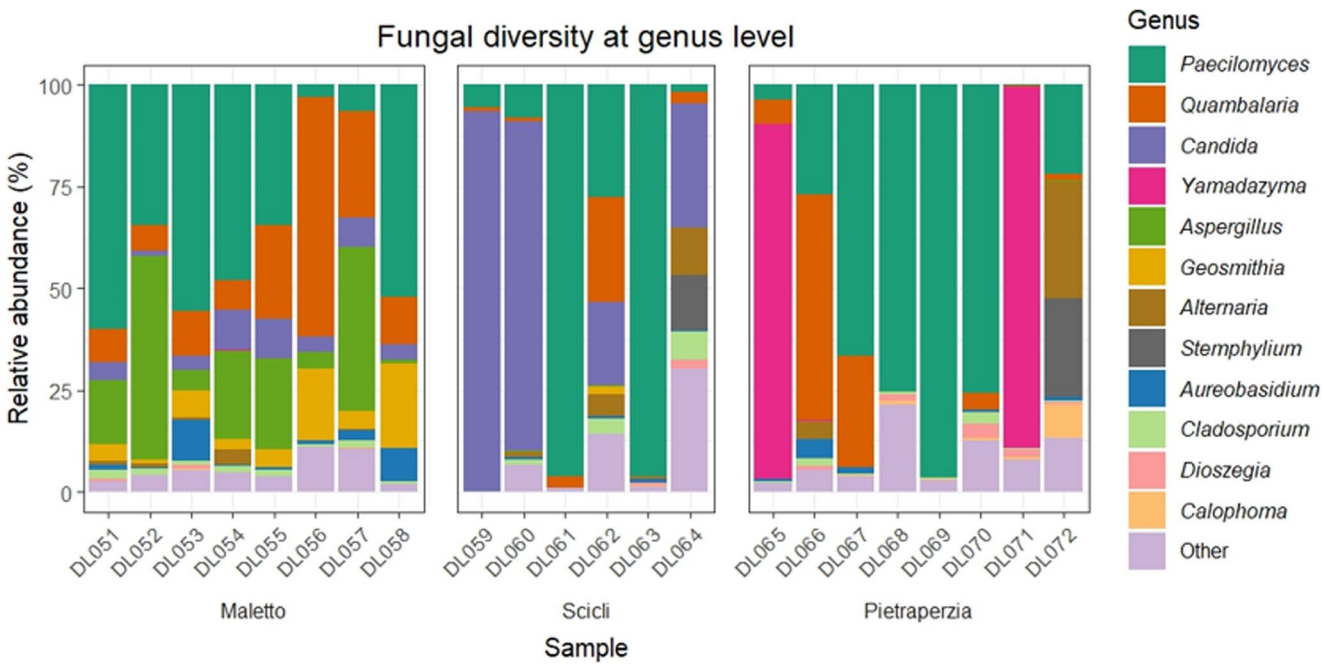

Fig. 6 Fungal diversity and relative abundance associated with *Scolytus amygdali* adults detected by the culture independent method. Genera under the detection threshold of 0.5% relative abundance are combined in "Other"

approach offered complementary taxonomic resolution, enabling the characterization of the culturable mycobiome to the species level, as detailed below.

Our findings revealed a common association of fungal species belonging to the *Geosmithia* genus with *S. amygdali*. In particular, *Geosmithia* spp. were isolated from all sampling sites and all tested organic material, except from wood lesions in a single sampling site (i.e., Pietraperzia). *Geosmithia* includes both generalist species reported in multiple beetle-host combinations or as plant endophytes and specialist species associated with insect vectors sharing host plants of the same family (Kolarík et al. 2004; Pitt and Hocking 2009; McPherson et al. 2013; Kolařík and Hulcr 2023). More importantly, the genus *Geosmithia* includes bark beetle symbiotic species that are tightly associated with their beetle hosts and benefit them nutritionally (Kolařík and Hulcr 2023).

To our knowledge, this is the first study reporting *Geosmithia* spp. consistently associated with *S. amygdali* in Mediterranean orchards, with the occurrence of two novel and still undescribed species (i.e., *Geosmithia* sp. 49 and *Geosmithia* sp. 50) and three already described species. In particular, our molecular characterization revealed *G. omnicola*, *G. pulverea* and *G. xerotolerans* as part of the *S. amygdali* mycobiome. Specifically, *G. xerotolerans* is known to be a cosmopolite fungus (Crous et al. 2018). It was isolated from galleries of *Phloeosinus* sp. in China (Zhang et al. 2022) and from many bark beetles in the Mediterranean and USA (Kolařík et al. 2007, 2017; Huang et al. 2017, 2019; Costanzo et al. 2026). Similarly, *G. omnicola* was isolated from beetles, galleries and/or surrounding, including *Scolytus* spp., in temperate Europe, Eurasia, Mediterranean area and South Africa (Kolařík et al. 2007, 2008, 2017; Pepori et al. 2015; Strzałka et al. 2021; Aylward et al. 2024; Costanzo et al. 2026). Finally, *G. pulverea* was found in association with bark beetles, such as *Scolytus* spp. and *Cryphalus* spp. in China, temperate Europe and USA (Kolařík et al. 2007, 2008; Strzałka et al. 2021; Zhang et al. 2022; Costanzo et al. 2026). Regarding the two novel species, i.e., *Geosmithia* sp. 49 and *Geosmithia* sp. 50, although molecularly characterized, their formal morphological descriptions is beyond the scope of this study and will be part of a dedicated taxonomic study.

Geosmithia spp. are stable and often dominant symbionts of many bark beetles (Kolařík and Hulcr 2023). Ambrosial, i.e., nutritional spore producing, *Geosmithia* are mutualistic, since they provide nutrition to the beetle hosts (Kolařík and Kirkendall 2010), but whether non-ambrosia *Geosmithia* spp. provide similar benefit is still not clear. Many *Geosmithia* spp. can degrade woody compounds, and others are able to use uric acid and beetle waste products as nitrogen sources (Veselská et al. 2019; Kolařík and Hulcr 2023). Moreover, *Geosmithia* spp. can synergize beetle aggregation via volatiles or interact with other fungi through mycoparasitism and antibiosis (Blood et al. 2018; Pepori et al. 2018, 2025; Hadj Taieb et al. 2019). Nevertheless, the nature of the association between *G. xerotolerans*, *G. pulverea*, *G. omnicola*, *Geosmithia* sp. 49, and *Geosmithia* sp.

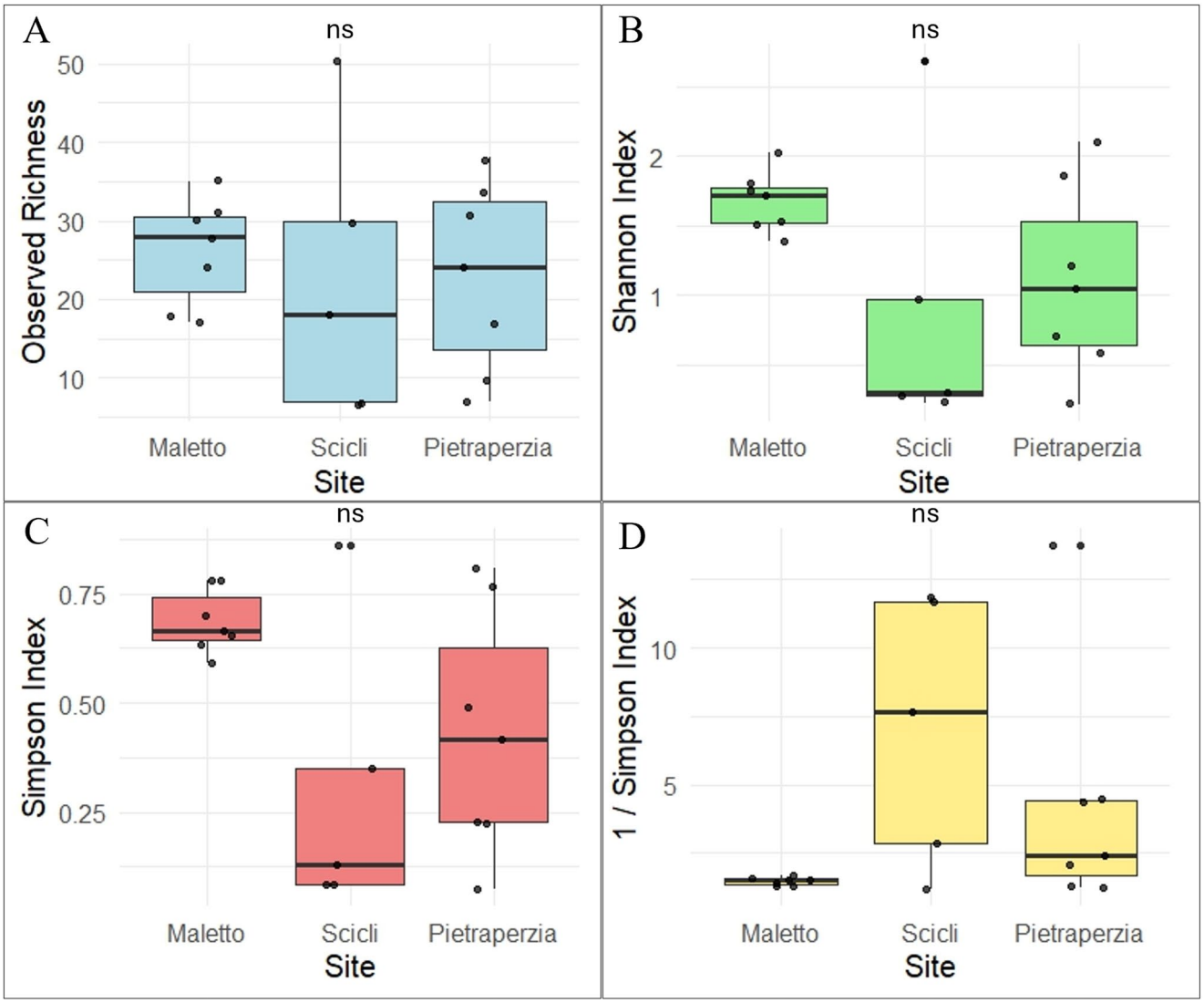

Fig. 7 Alpha diversity indexes representing the fungal assemblage richness and diversity from *Scolytus amygdali* among sampling sites. (**A**) Observed richness, (**B**) Shannon diversity, (**C**) Simpson and (**D**) Simpson's reciprocal dominance

50 with *S. amygdali* in infested trees of the investigated area is largely unknown.

In terms of pathogenicity, some *Geosmithia* can cause discoloration of the phloem around beetle galleries, but most species are not phytopathogenic on their own. An exception is *G. morbida*, vectored by *P. juglandis*, responsible for TCD on *Juglans nigra* L. (Kolařík et al. 2011; Veselská et al. 2019; Kolařík and Hulcr 2023). On the other hand, other *Geosmithia* species such as *G. omnicola* and *G. pulverea* showed no evidence of pathogenicity via seedling inoculations (Strzałka et al. 2021; Meshram et al. 2022). A few *Geosmithia* species induced phloem necrosis in *Pistacia vera* L. excised shoots, as for *G. omnicola* and *G. pallida*, the latter phylogenetically close to *G. pulverea* within the *G. pallida* species complex (Hadj Taieb et al. 2019; Zhang et al. 2022). However, since this approach does not fully replicate real-field conditions, additional trials using living plants in diverse environments are recommended.

In the present study, *Geosmithia* isolates were absent from only one almond orchard (i.e., Pietraperzia) when isolating from necrotic lesions. This result may be attributed to a low abundance of these fungi, dropping below the threshold of detectability of the culture-dependent method due to competition with other faster-growing fungi (Costanzo et al. 2026). In addition, *Geosmithia* isolates were not detected from *S. amygdali* through high-throughput sequencing analysis in the same site. However, given the consistent isolation of *Geosmithia* spp. from beetle adults from the same site on artificial media, this should not be considered as a real absence. Metabarcoding results may be strongly biased, which depends on the fungal taxon and the primers used. It is already well known that certain bark beetle associated

fungi (Ophiostomatales, *Geosmithia*) are underrepresented in ITS amplicon sequencing due to incomplete sequence databases and amplification biases (Hulcr et al. 2025).

Among the most occurring fungal taxa, *Paecilomyces* spp. were also consistently isolated from all sampling sites, and using both methodological approaches. The present study suggests that *Paecilomyces* spp. can colonise wood lesions, beetle bodies and galleries within the host plant-beetle system. Based on our findings, showing a consistent co-occurrence, *P. lecythidis* may represent a stable and mutualistic associate of *S. amygdali*, and his functional role in the bark beetle bio-ecology should be further investigated. To date, *Paecilomyces*-bark beetle associations have been poorly explored. Landa et al. (2001) found the entomopathogenic fungi *Cordyceps farinosa* (Holmsk.) Kepler, B. Shrestha & Spatafora (formerly *Paecilomyces farinosus* (Holmsk.) A.H.S. Br. & G. Sm) and the *Cordyceps fumosorosea* (Wize) Kepler, B. Shrestha & Spatafora (formerly *P. fumosoroseus* (Wize) A.H.S. Br. & G. Sm.) among the most occurring in *Ips typographus* L. adults in Czech Republic (Kepler et al. 2017). *Paecilomyces* spp. were also associated to *Scolytus kirschii* Skalitzky on *Ulmus minor* Mill. in Iran (Alizadeh et al. 2024), and to *Ips cembrae* Heer in Poland (Jankowiak et al. 2007), as well as isolated from dead *Hylesinus* (=*Chaetoptelius*) *vestitus* (Mulsant & Rey) individuals in Tunisia (Hadj Taieb et al. 2019).

Paecilomyces spp. play an important role as endophytes, offering various benefits for plant growth and development. Indeed, these fungi can have high efficacy for the control of agricultural pests and plant pathogens both in orchards and for protected crops as in the case of antagonistic species (i.e., biocontrol agents) (Chhipa et al. 2024; Shi et al. 2025).

However, the genus *Paecilomyces* includes also species known as plant pathogens causing dieback, decline and cankers on a wide range of fruit and forest trees (Alizadeh et al. 2000; Ghelichi et al. 2012; Sabbagh and Khosravi Moghaddam 2016; Mirabdollahi Shamsi et al. 2019; Hadj Taieb et al. 2019; Rostami and Jamali 2022; Goudarzi et al. 2024). Here, we provide the first report of *P. lecythidis* being isolated from woody necrotic lesions on almonds. Nevertheless, additional studies should assess its pathogenicity on specific host plants. Several studies report *P. maximus* C. Ram and *P. formosus* Urquarth, the two closest species to *P. lecythidis*, as tree pathogens (Heidarian et al. 2018; Ozan et al. 2022; Ören et al. 2023; Rostami and Jamali 2023). Specifically, *P. maximus* is reported as agent of dieback and canker in Turkey on pistachio and apricot (Ozan et al. 2022; Ören et al. 2023). In particular, *P. formosus* s.l was firstly suggested to be a species complex consisting of three taxa, *P. formosus* s.s., *P. lecythidis* and *P. maximus*, not distinguishable by morphological characteristics (Samson et al. 2009; Heidarian et al. 2018; Houbraken et al. 2020). More recently, Urquhart and Idnurm (2023) reported *P. formosus* [MB#846977, CBS 990.73B] as clearly distinct from its most closely related species *P. lecythidis* [MB#335530, CBS 372.70] and *P. maximus* [MB#335531, CBS 371.70] through whole genome comparisons. Nevertheless, the *P. maximus* isolates of the above-mentioned studies (Ozan et al. 2022; Ören et al. 2023) have been compared exclusively by ITS (FJ389927 and FJ389926) and β-tub (MF175900) sequences that, according to the updated phylogenetic analysis, belong to *P. lecythidis*, suggesting no discrimination between *P. maximus* and *P. lecythidis* in these studies.

Similar findings emerge from studies reporting *P. formosus* as dieback agent or detected from wood-boring beetles associated with oak dieback and decline (Rostami and Jamali 2023; Alizadeh et al. 2024; Ghaderi et al. 2025), or *Paecilomyces* spp. as disease agents on pistachio and other trees (Heidarian et al. 2018). However, according to the updated phylogenetic analyses, the actual tree pathogen of pistachio and oak trees could be *P. lecythidis* (CBS 372.70).

Outcomes of the culture dependent approach show a wide yet discontinuous distribution of *Quambalaria cyanescens* among sampling sites. This pattern could be attributed to a less stable association with *S. amygdali* than that observed for other fungal taxa, to the suppression of this slower-growing fungus by other fast-growing fungi on the artificial media, and/or to the difficulty to detect a low abundance fungus (Costanzo et al. 2026). However, data obtained from high-throughput sequencing revealed a consistent presence of *Quambalaria* on beetle adults, strengthening that this fungus may represents a common component of the *S. amygdali* mycobiome.

Quambalaria cyanescens occupies several ecological niches, including insects and plants (de Beer et al. 2006). Here, we report *Q. cyanescens* associated with *S. amygdali* infesting almond trees in Italy, similar to what previously observed in Syria (Kolařík et al. 2006). Interestingly, this fungus has been frequently associated with bark beetle feeding on hardwood host trees (Kolařík et al. 2006). However, the way how heterobasidiomycete fungi interact with beetles and other fungi as part of their mycobiome remains unclear, even though it has been suggested that galleries of bark beetles are a niche for many of them (Kolařík et al. 2006). *Q. cyanescens* could be a mycoparasites of ambrosia and blue-stain fungi that are closely associated with the beetles (Six 2005). Preliminary trials showed no mycoparasitic activity by *Q. cyanescens* (Kolařík et al. 2006). Later, Stodůlková et al. (2015) found species-specific in vitro antibiotic effects on fungi growing in bark beetle galleries, including *Geosmithia* spp., *Graphium* sp., and on the entomopathogen *Beauveria bassiana (Bals.-Criv.) Vuill.* The potential entomopathogenicity of *Q. cyanescens* remains poorly investigated, but its common isolation from living

adults or larvae suggests it as harmless to beetles (Kolařík et al. 2006).

Previous studies reported *Q. cyanescens* on symptomatic and/or healthy tissues of *Eucalyptus pauciflora* Sieber ex Spreng. (de Beer et al. 2006) and *Corymbia* spp., and subsequent pathogenicity trials confirmed *Q. cyanescens* as non-pathogenic to *Corymbia* spp. (Paap 2006; Paap et al. 2008), suggesting it as saprophyte of various plants (de Beer et al. 2006; Kolařík et al. 2006). Antimicrobial activity of *Q. cyanescens* endophytic isolates was instead observed in vitro (Dolatabad et al. 2017; Preto et al. 2017). Middelhoven et al. (2000) observed the *Q. cyanescens* ability to degrade typical plant metabolites and plant cell wall constituents, suggesting living or decaying tissue as natural habitat for the fungus. More recently, it was isolated from plants showing grapevine trunk disease in Iran, but pathogenicity tests revealed a low aggressiveness (Shekariesfahlan et al. 2025). In addition, it has been reported as a pathogen responsible for grapevine decline in Iran (Narmani and Arzanlou 2019) and for causing Grapevine Trunk Diseases (GTDs) on grape propagation material in Italy (Mattia et al. 2025). It was also hypothesized as part of the GTDs complex in the Americas (Travadon et al. 2022; Argüelles-Moyao et al. 2024).

As known, the characterization of fungal communities in wood-boring insect systems can be significantly influenced by the isolation methodology employed (Hulcr et al. 2025). In the present study, when isolating fungi from internal wood necrotic lesions, we prioritized the recovery of species actively colonizing wood tissues, including potential phytopathogens. By utilizing surface sterilization (with sodium hypochlorite), we aimed to eliminate ubiquitous saprophytes and fast-growing environmental contaminants, as well as secondary colonizers associated with advanced decay. While this procedure may selectively exclude certain superficial or sensitive taxa, it was essential to ensure that the resulting isolates originated from within the actively progressing necrotic lesions. On the other hand, when isolating fungi from active galleries, the stereomicroscope-guided mycelial scraping was employed to maximize recovery of the primary mycobiome members inhabiting beetle gallery systems. Collectively, these methodological choices, together with isolations from the insect body, allowed the isolation of functionally relevant community members within the insect ecological niches.

Although the three fungal genera consistently associated in the present study with the almond bark beetle are *Geosmithia*, *Paecilomyces* and *Quambalaria*, our results from metabarcoding show that ASVs of other genera are part of the *S. amygdali* fungal community. Interestingly, we isolated *Candida*, *Yamadazyma*, *Aspergillus* and *Cladosporium* from adults of this bark beetle, corroborating previous findings from similar studies on other bark beetle species (Davydenko et al. 2017; Masuya et al. 2019; Vazquez-Ortiz et al. 2022; Pineda-Mendoza et al. 2024; Barta et al. 2025). *Candida* is one of the dominant members of the gut core microbiome of bark beetles, together with *Danielia*, *Cyberlindnera*, *Yamadazyma* and others (Pineda-Mendoza et al. 2024; Vazquez-Ortiz et al. 2025). Saccharomycetales, including *Candida* and *Yamadazyma* yeasts, have been suggested to be involved in nutrient acquisition, detoxification processes, and microbial interaction within beetle galleries. In addition, they contribute functionally to metabolic complementation and chemical communication (Six and Klepzig 2021). Some yeast species may also be involved in the biosynthesis of pheromones or in mediating metabolite signalling, thus affecting key behavioural traits in beetles, such as aggregation and reproduction (Davis 2015; Hofstetter et al. 2015).

The outcomes of the present study underscore the importance of combining culture-dependent and culture-independent approaches when investigating the mycobiome of bark beetles. For example, isolates belonging to all the three most frequent fungal genera (*Geosmithia*, *Paecilomyces* and *Quambalaria*) were detected using both methods, although some taxa were not detected via metabarcoding from beetle adults and were only identified through culturing samples from the same site, as previously outlined. This finding further supports how integrating these methods helps to overcome the inherent limitations of a single approach, increasing the likelihood of capturing the full diversity of the beetle-associated fungal community, as extensively reviewed in Costanzo et al. (2026).

Overall, this study lays the groundwork for future research aimed at further investigating the role and variability of the *S. amygdali* mycobiome in other regions. More importantly, the occurrence of potential phytopathogenic species highlights the need of specific studies directly focusing on the complex interactions among the almond bark beetle and components of its mycobiome with almond trees, as well as the mechanisms by which beetles can facilitate fungal transmission or act as vectors.

6 Conclusion

In the present study, we assessed the variability of the fungal community associated with the almond bark beetle in southern Mediterranean orchards, emphasising the importance of integrating culture-dependent and culture-independent approaches. Our results provide the first comprehensive overview of the fungal taxa primarily associated with *S. amygdali*, including *Paecilomyces*, *Quambalaria* and *Geosmithia*. Furthermore, our metabarcoding outcomes offer new insights into the potential gut microbiome of these

bark beetles, including *Candida* and *Yamadazyma* yeasts. Future research is needed to explore potential geographical and host plant-specific differences in the *S. amygdali* mycobiome. The further geographic expansion of *S. amygdali*, especially into regions outside of its native distribution, could pose a significant threat to stone fruit production, as has been observed with almonds in some areas of the Mediterranean region. A deeper understanding of these associations is indeed a vital step towards developing sustainable and efficient management strategies that target bark beetles and/or their potentially vectored fungi.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13199-026-01154-6.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mariangela Benedetta Costanzo. The first draft of the manuscript was written by Mariangela Benedetta Costanzo and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability All data supporting the findings of this study are available on request.

Declarations

Competing interests The authors declare no competing interests.

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