Journal of Plant Diseases and Protection (2026) 133:74
https://doi.org/10.1007/s41348-026-01268-5

ORIGINAL ARTICLE

# First report and pest risk analysis of the ambrosia beetle associated fungus *Graphium euwallaceae* in Germany

**Gritta Schrader**[1] · **Katja Boldt-Burisch**[2] · **Björn Hoppe**[1] · **Clovis Douanla-Meli**[1]

Received: 8 September 2025 / Accepted: 30 March 2026 / Published online: 15 April 2026

## Abstract

The fungus *Graphium euwallaceae* is associated with ambrosia beetles (*Euwallacea* sp.) and has been found in e.g. avocado trees, box elder, and evergreen maple. It is known to occur in California, Vietnam, and Australia. In early 2025, the fungal isolate AGQMy-2025-02 was obtained from a borehole in *Barringtonia acutangula* (Lecythidaceae) in connection with an outbreak of the *Euwallacea* beetle in a tropical greenhouse in Germany and suspected to be *G. euwallaceae*. A combination of morphological and molecular methods employing barcoding across four gene regions (ITS, TEF1-α, SSU and LSU) and phylogenetic reconstruction based on ITS and TEF1-α was used to identify this isolate as *G. euwallaceae*. This is apparently the first detection of the fungus in Europe. There is no information, that *G. euwallaceae* is regulated as a quarantine pest in any plant health regulation, and it is so far neither listed in the annexes to Regulation (EU) 2019/2072 nor by the European and Mediterranean Plant Protection (EPPO). This paper describes the diagnosis and presents a pest risk analysis for this fungus.

**Keywords** Ambrosia fungi · Diagnosis · *Euwallacea* sp. · Pest risk analysis · Plant health

## Introduction

Ambrosia beetles belong to the weevil subfamilies Scolytinae and Platypodinae (Coleoptera, Curculionidae) that live in close nutritional symbiosis with ambrosia fungi (Osborn et al. 2023). These beetles bore galleries into the xylem (woody tissue) of their host plants, where they lay their eggs and where larval development takes place. Unlike phytophagous beetles, ambrosia beetles and their offspring do not feed directly on the xylem, but on cultivated symbiotic fungi, the so-called "brood fungi". This highly specialized relationship enables the beetles to reproduce successfully even in dead or physiologically weakened wood and plays a crucial role in their ecological adaptability.

One of the most economically and ecologically important species is *Euwallacea fornicatus* (Eichhoff 1868). This beetle species is native to Southeast Asia, but has spread to numerous other regions in recent decades, including North America, Israel, South Africa and parts of Europe (e.g. Bierman et al. 2022 and 2023, Schuler et al. 2023; Goldarazena et al. 2025). In Europe, occurrences, with one exception in Spain, have so far been documented in controlled environments such as botanical gardens and tropical greenhouses (Schuler et al. 2023; Goldarazena et al. 2025). As with ambrosia beetles in general, spread primarily occurs through the transportation of infested plants or wood products (Dzurenko and Hulcr 2022). Due to the close symbiosis with fungal species, of which some have been identified to be pathogenic, an infestation can lead to the death of the infested branches or entire plants of a large number of host plants. The larval development of *E. fornicatus* depends entirely on the availability of the associated fungi. These fungi are actively transported by the beetle and carried in special structures, the mycangia (Hulcr and Skelton 2023), between the mandibles or in other parts of the body. In the newly created feeding galleries, the fungi are introduced into the xylem, where they spread and break down the cell walls. This creates an easily accessible food source for the

---

✉ Gritta Schrader
gritta.schrader@julius-kuehn.de

[1] Julius Kühn Institute (JKI) – Federal Research Centre for Cultivated Plants, Institute for National and International Plant Health, Braunschweig, Germany

[2] Phytopathology, State Office for Rural Development, Agriculture and Land Reconciliation (LELF), Zossen, Germany

larvae. Numerous studies have shown that *E. fornicatus* is not restricted to a single fungal partner, but lives in association with a fungal community (Freeman et al. 2016). It has been associated in particular with the fungus *Fusarium euwallaceae* Freeman, Z. Mendel, T. Aoki & O'Donnell (synonym: *Neocosmospora euwallaceae*). Both, the beetle and the fungus are listed as quarantine pests in the European Union (EU 2019). *Fusarium euwallaceae* belongs to a group of species that has been detected in several geographical regions associated with *Euwallacea* species and is in many cases responsible for the occurrence of disease symptoms on host plants, such as wilting, bark discoloration or the death of entire plant parts. The pathogenicity of the associated fungi, combined with the transportation capacity of the beetles, results in considerable economic damage in affected regions, particularly to fruit trees, ornamental plants and forestry-relevant woody plants (EPPO 2025a).

In addition to *F. euwallaceae*, several studies have documented other fungal species associated with *E. fornicatus*, including representatives of the genera *Graphium*, *Acremonium*, *Paracremonium*, and other saprotrophic or opportunistic fungi (Carrillo et al. 2016; Freeman et al. 2016; Lynch et al. 2016). The genus *Graphium* includes filamentous ascomycetes that are often associated with insects and sometimes occur as secondary symbionts in ambrosia beetle communities. In many cases, the role of these fungi within the symbiosis is not yet fully understood. They could act as an additional food source, as competitors to primary brood fungi or as colonizers of weakened tissue. However, a new study by Cruz et al. (2025) showed that not all the ambrosia associated fungi might have the same positive symbiotic effect on beetle nutritional support. Cruz et al. (2025) showed that the larvae of the *Euwallacea fornicatus* complex have a strong positive nutritional relationship with *Fusarium* species but not with the fungi of the genera *Graphium* (Microascales), *Paracremonium* and *Acremonium*, which are therefore considered as auxiliary fungi. In later stages of the life cycle of an ambrosia beetle though, Freeman et al. (2016) found, that non-sclerotized adults preferred *Graphium euwallaceae* as a food source.

In this paper, the first finding of *Graphium euwallaceae* M. Twizeyimana, Lynch and Eskalen, 2016 (Ascomycota, Microascales, Graphiaceae) in association with *E. fornicatus* in a German Tropical greenhouse detected by multilocus phylogenetic analysis is reported. This fungus was originally described in California, where it was isolated together with *F. euwallaceae* and other fungal species from plants infested by *E. fornicatus* (Lynch et al. 2016). Many of the outdoor locations where *G. euwallaceae* was detected, including California, Vietnam and Australia (Lynch et al. 2016; Trollip and Carnegie 2025) have tropic/subtropic climatic conditions, suggesting that the natural habitat of the fungus is in the tropical/subtropical region. The exact ecological function of *G. euwallaceae* has not yet been conclusively investigated, but it is assumed a secondary symbiont, possibly in the initial colonization phase of the wood or in competitive relationship or coexistence with *Fusarium* species. Following this first finding of *G. euwallaceae* in Germany, an initial pest risk analysis (PRA) had to be conducted (Schrader et al. 2024), as the Julius Kühn Institute (JKI) serves as the competent plant health authority for Germany to evaluate biological, scientific, and economic evidence to determine whether a newly identified organism harmful to plants is a quarantine pest, whether it should be regulated, and to identify appropriate phytosanitary measures to mitigate risks of entry, establishment, and spread.

Against the background of increasing global trade and increasing mobility of traded plant material, the understanding of such insect-fungus symbioses is gaining in importance. The transportation of infested plants can introduce both the beetle and its symbiotic fungi into new regions.

## Materials and methods

### Sampling

In January 2025, a symptomatic branch (Fig. 1) from approx. 1.5 m height was taken from *Barringtonia acutangula* (L.) Gaertn., common name Indian Putat, in a tropical greenhouse in Germany. Symptoms were small, perpendicular boreholes of approx. 1 mm diameter, and slight elevations in the cambium, partly surrounding the branch. Bark was still present and did not peel off. Due to the small boreholes caused by the vector and symptoms of infestation, an infection with *Fusarium euwallaceae*, associated with the occurrence of the ambrosia beetle, was initially suspected, which had previously been detected in *Barringtonia acutangula* and other trees close by.

### Identification of the fungus

The fungus can be identified using morphological and molecular methods (e.g. Lynch et al. 2016). In addition to the morphological examination, a multilocus sequence typing (MLST) approach or the concordance of multiple genealogies (based on ITS, EF 1-α, cmdA, acl1, tub2, 28 S, and rpb2 genes) was used to confirm the species identification.

### Morphological characterisation

From the symptomatic branch, nine fragments from the wood surface were sterilized with 70% Isopropanol for 30 s, then rinsed three times with sterilized water each for

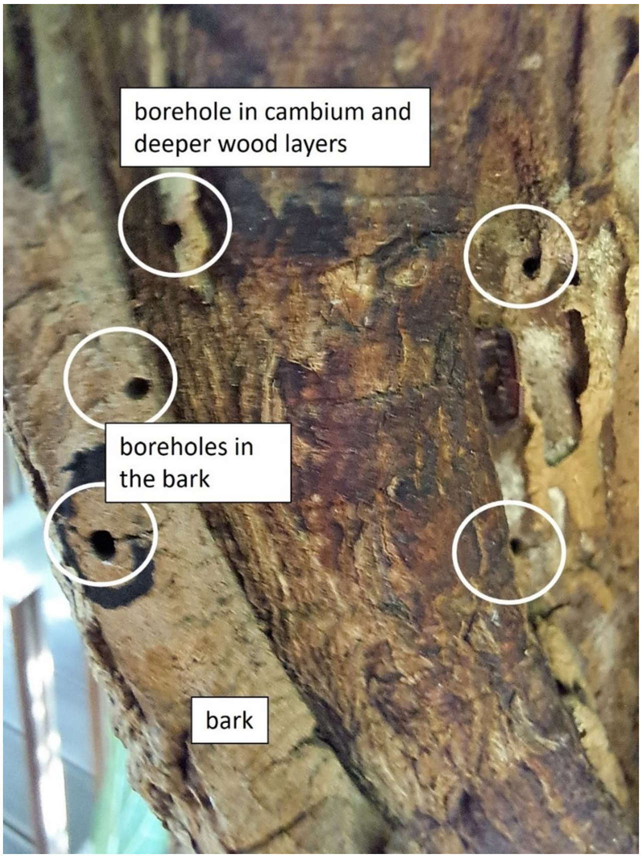

**Fig. 1** Boreholes in the bark of *Barringtonia acutangula* created by the vector *Euwallacea* sp. (Photo: Toralf Pfannenstill, LELF)

1 min and air dried on filter paper. Sterilized wood fragments were cut into smaller pieces on the clean bench and then transferred to synthetic nutrient-poor agar (SNA; Sifin Diagnostics GmbH, Germany) plates, incubated for 2 weeks at 24 ± 2 °C under daylight conditions.

The fungus was then identified based on morphological characteristics described in Lynch et al. (2016) and Okada et al. (1998, 2000).

## Molecular identification

A molecular investigation based on DNA barcoding and phylogenetic reconstruction was performed to corroborate the morphological characterisation of the isolate AGQMy-2025-02. Genomic DNA was extracted from colonies grown for 2 weeks at 22 ± 2 °C under daylight conditions on SNA, using the innuPREP Virus DNA/RNA Kit KFml (Analytik Jena, Germany) and KingFisher ml (Fisher Scientific GmbH, Schwerte, Germany) following the manufacturer's protocol. Four gene regions (*ITS*, *EF1-α*, *SSULSU* were amplified using the primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) for *ITS*, EF1-728 F and EF2R (Jacobs et al. 2004) for *EF1*-α, NS1, NS3, NS4 and NS8 (White et al. 1990) for *SSU*, LR0R and LR5 (Vilgalys and Hester 1990) for *LSU*. The PCR was performed in a 25 µL reaction mixture. PCR parameters for the *ITS*, *SSU* and *LSU* genes were 3 min for 94 °C followed by 35 cycles of 30 s at 94 °C, 30 s at 55 °C and 1 min at 72 °C, and finally one cycle of 7 min at 72 °C, and for the *EF1*-α were 5 min at 94 °C, then 40 cycles of 45 s at 94 °C, 30 s at 52 °C and 1 min at 72 °C, followed by a final cycle of 6 min at 72 °C. The amplicons were purified with QIAquick PCR Purification Kit (QIAGEN GmbH, Hilden, Germany) and then sequenced in both directions using the corresponding PCR primers by Macrogen Europe (Netherlands). Raw nucleotide sequences were edited in MEGA v. 7 (Kumar et al. 2016).

The generated sequences were used for a BLASTn analysis in the NCBI database to search for highly similar sequences. The matching sequences, including those from the type material, were then retrieved for phylogenetic analysis. The alignments in the online version of MAFFT v.7 (Katoh et al. 2019) were further optimized manually using MEGA v.7. Trees were generated using the maximum likelihood (ML) and maximum parsimony (MP) algorithms. ML was performed using the online version of PhyML 3.1 (Dereeper et al. 2008; https://www.phylogeny.fr/) and MP was performed using PAUP* 4.0a169 (Swofford 2003) respectively. Clade stability was assessed (Rambaut 2012) using 1000 bootstrap replicates. Trees were visualized and edited in FigTree v.1.4.0.

## Pest Risk Analysis (PRA)

To assess the risk of the fungus being introduced into new areas — including its potential for entry, establishment, and spread, as well as the potential impacts — a short PRA (Express PRA) was conducted (for more background on the conduction of PRA, see Schrader et al. 2024). This PRA follows the standards of the International Plant Protection Convention (IPPC) and the European and Mediterranean Plant Protection Organization (EPPO), and complies with the relevant European Union regulations (IPPC 2001, EPPO 2024, EU 2016). The following key aspects were addressed in the PRA: (1) Biology of the fungus: An overview of the life cycle and biological properties of the organism; (2) geographical distribution of the fungus and areas of infestation: A summary of the current distribution and areas already infested; (3) host plants and their presence in the PRA area: Identification of susceptible host plant species and assessment of their occurrence in the PRA area (Germany and the EU); (4) symptoms of infestation: Description of the typical signs and symptoms of infection; (5) climatic suitability: Comparison of climatic conditions in the fungus' established range with those in the PRA region to assess suitability for establishment; (6) pathways of introduction

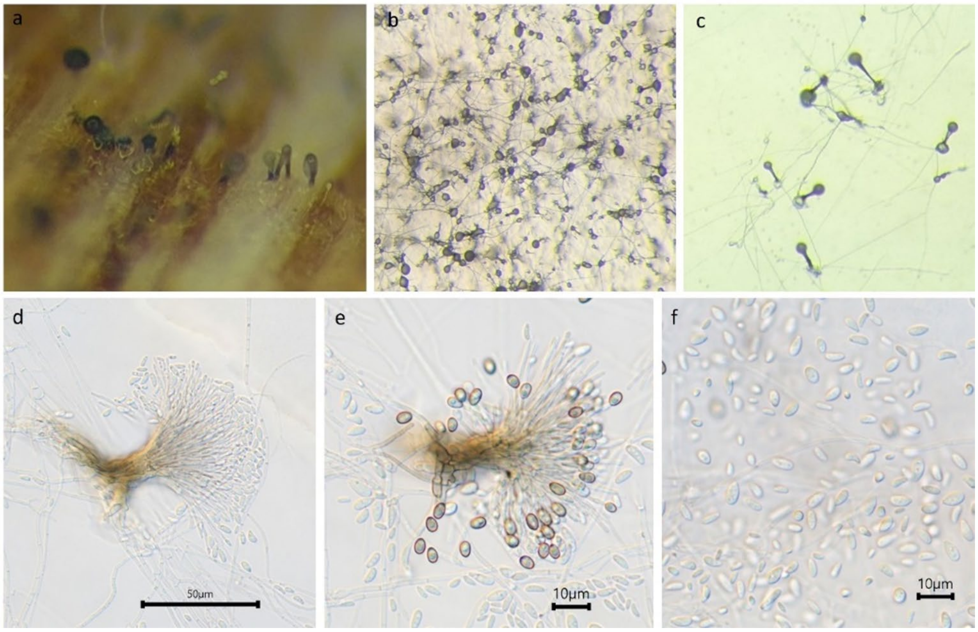

**Fig. 2** *Graphium euwallaceae*. Synnema growing out of the symptomatic wood on SNA (**a**), weave of hyphae and synnema on SNA (**b**) synnema on SNA (**c**), synemma with thin conidia (**d**), conidiougenous apparatus and round and dark pigmented conidia and thin conidia (**e**), thin conidia (**f**) of 2 weeks old cultures (Photos: Dr. Katja Boldt-Burisch, LELF)

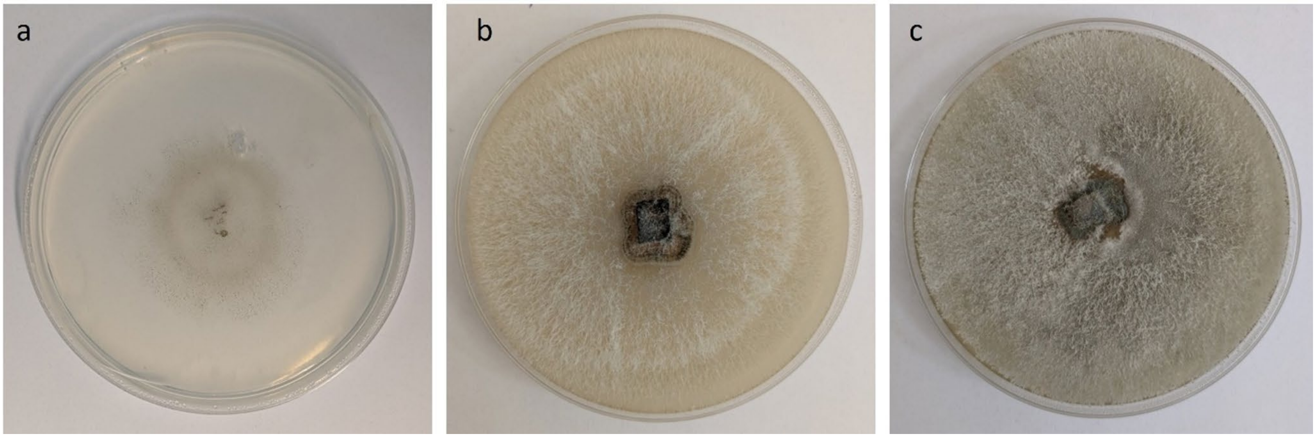

**Fig. 3** Colony morphology of *Graphium euwallaceae*: upper surface of 2-week-old cultures on SNA (**a**), MEA (**b**) and PDA (**c**) (Photos: Dr. Katja Boldt-Burisch, LELF)

and spread: Analysis of natural and human associated routes by which the fungus could enter and spread; (7) known impacts in areas where the fungus is present: Assessment of known economic, environmental, and social impacts in these areas, as well as potential impacts in new areas; (8) expected establishment and spread in Germany and the EU: taking into account the current climatic conditions and the potential pathways; (9) Expected impacts in Germany and the EU: based on information about spread and available host plants; (10) control of the fungus: assessment of the likelihood and feasibility of successful eradication following introduction.; (11) diagnosis.

This comprehensive approach ensures that all the factors that influence the risk posed by the fungus are systematically considered and assessed in accordance with established international and regional guidelines.

## Results

### Morphological identification

*Graphium euwallaceae* was morphologically identified. The fungus produced hyaline, highly septate hyphae, dark pigmented synnema and thin and round pigmented conidia on the wood and on SNA medium after 2 weeks (Fig. 2a-f).

Culture characteristics: Colony diameters were 3.7–4.5 cm on SNA, and 7.0–8.5 cm on PDA and 9 cm on MEA after 2 weeks incubation at 24 ± 4 °C under daylight conditions (Fig. 3a-c).

The colonies on SNA were hyaline, later appearing partly slightly brownish due to a large amount of dark pigmented synnema between hyphae. Hyphae were appressed and immersed (Fig. 3a). Colonies on MEA were initially (age $<$ 7 days) whitish on the upper surface, after two weeks turning ivory-white and slightly fluffy, with rough and filamentous edges (Fig. 3b). Colonies on PDA were irregular to patchy greyish and fluffy on the upper surface (Fig. 3c). Colony colour on the reverse plates were grey for PDA and MEA with a darker grey on PDA.

## Isolate identification

In BLASTn analysis, the ITS sequence of AGQMy-2025-02 (500 bp) matched those of *G. jumulu* (GenBank accession no. PP992042) and *G. euwallaceae* (GenBank accession no. KF540225), with 100% (568/568) and 100% (555/555) similarity respectively. The *TEF1-α* sequence (500 bp) matched those of *G. euwallaceae* (GenBank accession no. PV033312 and PP712914) with a similarity of 100% (500/500 bp), and the next related species was *G. carbonarium* (GenBank accession no. MT625161 and MT625162) with 99% (493/500 bp) similarity. The LSU sequence (583 bp) matched those of *G. euwallaceae* (GenBank accession no. MT252037), *G. basitruncatum* (GenBank accession no. HG857747) and *G. carbonarium* (GenBank accession no. MH874834) with 100% (582/582 bp) similarity. The SSU sequence (2090 bp) matched those of *G. euwallaceae* (GenBank accession no. KF542895, KF542896, KM385422) with 99% (2089/2091), 99% (2089/2091), and 99% (2067/2068) similarity respectively.

As only a few *Graphium* species have *LSU* and *SSU* sequences available in GenBank, we have refrained from constructing trees for these regions. The *ITS* and, in particular, the *TEF1-α* regions are better suited to species grouping and delimitation in *Graphium*. The results of the sequence similarity comparison indicate that *G. euwallaceae*, *G. carbonarium*, *G. jumulu* and *G. kuroshium* are genetically very closely related. This is also reflected in single-locus trees (data not shown) and in the combined tree using *ITS* and *TEF1-α* (Fig. 4). The sequences of the four species are found to cluster in one clade, forming distinct groups that are, however, poorly resolved. This is consistent with the findings reported in previous studies (Li et al. 2024; Bangasu and Lee 2025). It was observed that, in all trees, the sequence of the isolate AGQMy-2025-02 consistently clusters with that of *G. euwallaceae*. This placement can be further substantiated by ecological evidence, as the beetle from which the isolate AGQMy-2025-02 was obtained could be identified as *Euwallacea fornicatus*.

## Pest Risk Analysis (PRA)

According to the procedure presented in Material and Methods, the following results were found:

### Biology of the organism

As mentioned above, *Graphium euwallaceae* is a xylem inhabiting ambrosia fungus that lives in obligate symbiosis with ambrosia beetles of the genus *Euwallacea*. The fungus produces conidia, which are transported by the beetles in the mycangia, which in this case is located in the head region of the insect. In ambrosia beetles of the subfamily Platypodinae, mycetangia are present in one or both sexes. However, in ambrosia beetles of the genus Scolytinae — including the *Euwallacea* species — only the females possess these structures. Adult female beetles act as vectors, transmitting the fungus within and between trees, by boring into the wood and creating new galleries. Once established, the fungus colonizes the xylem and absorbs the nutrients it needs to grow. The life cycle of the fungus is closely linked to that of its insect host. Larvae feed exclusively on the cultivated fungus until maturity. Fungal colonization is not limited to the area surrounding the galleries but can also extend into the surrounding wood tissue, thus increasing the infected area (Six 2012; Carrillo et al. 2014; Lynch et al. 2016; and references cited therein). Beetles of the genus *E. fornicatus* prefer to infest branches and twigs previously colonized by conspecifics. Compared to primary infections, reproductive success is significantly higher here (Mendel et al. 2017). This repeated colonization often results in the formation of large beetle-fungus colonies. The beetles repeatedly infest the same sections of the same tree until they become unsuitable for further fungal development. At this stage, the beetles search for new sections of the same tree or for new host trees (Hulcr and Skelton 2023).

### Geographical distribution of the fungus and areas of infestation

The fungus has so far been found in California, Vietnam, and Australia (Lynch et al. 2016; Trollip and Carnegie 2025). The discovery in a tropical greenhouse in Brandenburg appears to be the first detection in Europe.

### Host plants and their presence in the PRA area

*Euwallacea fornicatus* sensu lato is known to infest more than 200 host plant species. However, it remains unclear whether it is able of transmitting *G. euwallaceae* to all of these hosts, therefore this possibility cannot be excluded.

Lynch et al. (2016) detected *G. euwallaceae* in California sycamore (*Platanus racemosa* Nutt.), box elder (*Acer negundo* L.), avocado (*Persea americana* Mill.), and castor bean (*Ricinus communis* L.) in California, and in Vietnam in ear-pod wattle (*Acacia auriculiformis* A. Cunn. ex Benth.). Trollip and Carnegie (2025) found *G. euwallaceae*

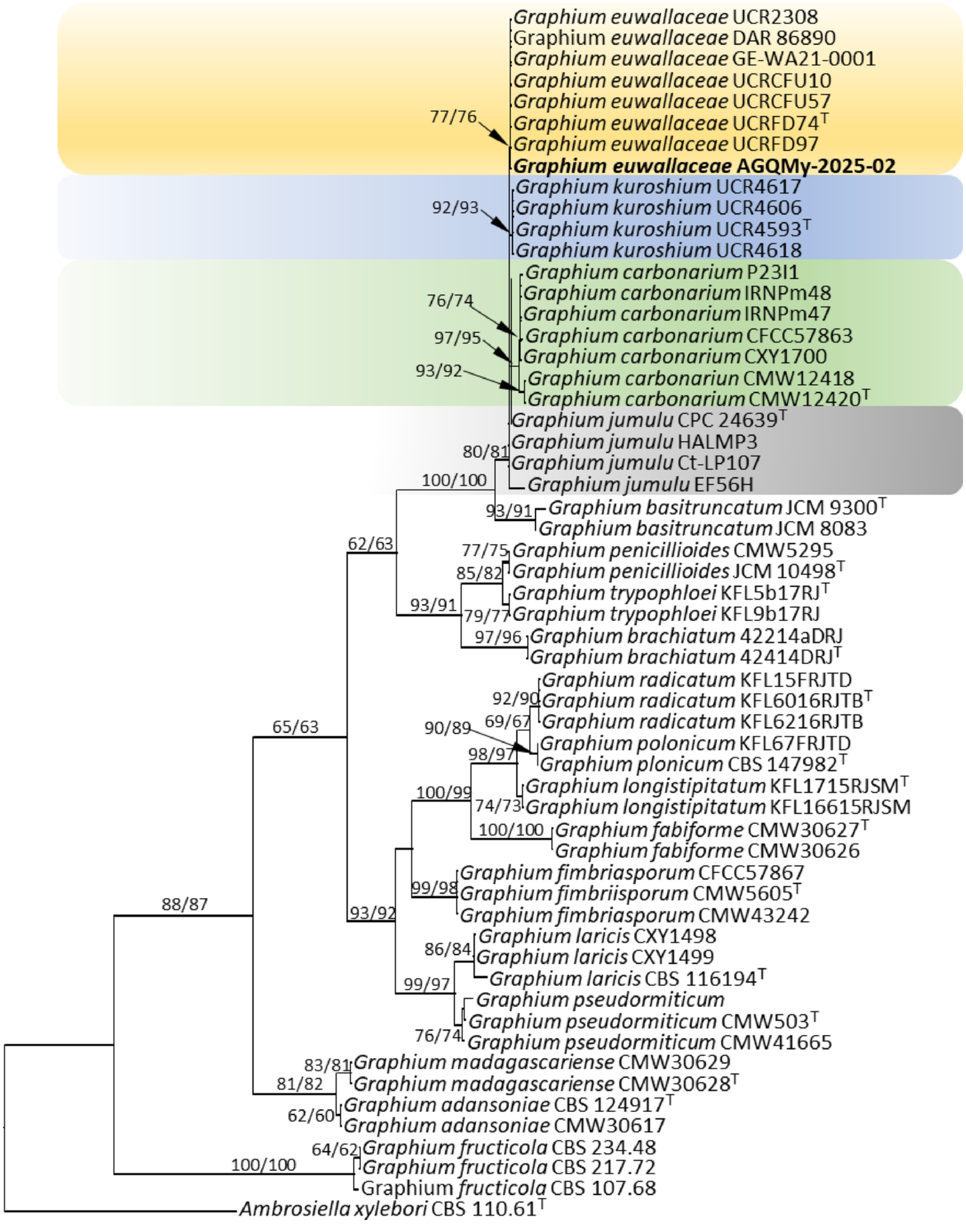

**Fig. 4** Phylogenetic tree based on maximum likelihood analysis of a concatenated *ITS and TEF1-α* sequence data showing the relationships among *Graphium* species. The numbers at each node represent the bootstrap values (>60%) obtained from 1,000 replicates using maximum likelihood and parsimony, respectively. *Ambrosiella xylebori* (CBS 110.61) was used as outgroup. The sequence generated in this study is in bold and T indicates the ex-type strain. Visualized and edited in FigTree v.1.4.0 (Rambaut 2012)

in evergreen maple (*Acer paxii* Franch.) in Sydney, Australia. In Germany, the fungus was detected in the paleotropical species *Barringtonia acutangula*.

According to GenBank entries by Lynch et al. (2016), the fungus has also been detected in the following host plants: *Acacia floribunda* (Vent.) Willd., *Erythrina atitlanensis Krukoff & Barneby*, and *Quercus agrifolia* Née. See also https://www.ncbi.nlm.nih.gov/nuccore/KF540221.1 and Na et al. 2018; Table 2.

Most of the above-mentioned host plants occur in the tropics and/or subtropics. Box elder is native to North America, but was introduced to Central Europe as early as the 19th century and is widespread in Germany (BfN 2023a). Castor bean is native to Northeast Africa and the Middle East, but is now found sporadically in Germany (BfN 2023b). *Acer paxii* is native to Central China and is not winter hardy in Central and Northern Europe, but is available from nurseries.

*Persea americana* is cultivated commercially in southern EU Member States. *Acer negundo* and *Ricinus communis* are also found in the EU. Since *A. paxii* is available in nurseries, it cannot be excluded that it may be found outdoors as an ornamental plant in particular in southern European Member States.

### Symptoms of infestation

Symptoms of trees heavily infested with beetles and fungi (not limited to *G. euwallaceae* alone) include wilting, leaf discoloration, trunk breakage, and tree death (Lynch et al. 2016; and studies cited therein).

### Climatic suitability

The fungus has so far been detected in California, USA, Vietnam, and Sydney, Australia. According to the climate classification following Köppen-Geiger, California mostly has a warm, rainy climate, the coldest month is usually between 18 and − 2 °C, the driest period is in summer, and the mean temperature of the warmest month is above 22°C. This classifies as "hot summer Mediterranean climate", Csa. Vietnam and Sydney are mostly subtropical. They have a warm, rainy climate, the coldest month is between 18 and − 2 °C, it is consistently humid, the mean temperature of the warmest month is above 22°C. This classifies as "humid subtropical climate", Cfa (Lynch et al. 2016; Köppen 1918; Kottek et al. 2006).

In southern, subtropical EU member states, particularly in areas with the Köppen-Geiger classification Csa and Cfa (Köppen 1918; Kottek et al. 2006; for classifications of Europe, see Macleod and Korycinska, 2019), the climate would most probably be suitable. The "hot summer Mediterranean climate" (Csa) is found primarily in Portugal, Spain, Italy (including Sicily), southern France (including Corsica), Greece, and small parts of Bulgaria, Croatia, and Cyprus. The subtropical "humid subtropical climate" (Cfa) is found in the north and on the Adriatic coast of Italy, in Slovenia and Croatia, in the border area between Romania and Bulgaria, and in a small part of northern Greece, in parts of southern France, in southern Hungary, and in northern Spain.

As the fungus was detected in *Barringtonia acutangula* in a tropical greenhouse, it is obvious that host plants are also present in protected cultivation with tropical/subtropical conditions. Since *Euwallacea* sp., the vector of *G. euwallaceae*, can infest a wide variety of host plants, it is assumed that there are additional host plants in protected cultivation. However, no concrete information is available on this.

### Pathways of introduction and spread

The fungus can be spread primarily through infected host plants for planting (e.g. from one tropical house to another) as well as via transport of infected wood and wood packaging material.

*Graphium euwallaceae* is spread over short distances by female ambrosia beetles (*Euwallacea* sp., see Biology of the organism). Whether the fungus can also spread independently from the beetles is unknown (Hulcr and Skelton 2023). Natural spread between greenhouses is rather unlikely.

### Known impact in infested areas

Fungi associated with ambrosia beetles block the vascular tissue in the xylem, thereby impairing the nutrient and water supply of their host plants. In heavily infested plantations of avocado trees, productive branches break more frequently. Generally, branches often break where beetle galleries are found. Both young and mature trees can die (Lynch et al. 2016; Mendel et al. 2012). In inoculation experiments, two-year-old avocado seedlings infected with *G. euwallaceae* showed lesions with an average length of 6.4 ± 2.8 cm (Na et al. 2018). The ultimate cause of tree death is most likely the combined effect of two factors: first, the slow but persistent accumulation of *Euwallacea* beetle galleries at the base of large branches and on the trunks of susceptible trees (Hulcr et al. 2017; Hulcr and Skelton 2023). Second, the localized pathogenicity of the associated fungus, as described for *Fusarium euwallaceae*. Together, these factors cause cambium necrosis, branch dieback, and ultimately tree death (Lynch et al. 2016).

### Expected establishment and spread in Germany and the EU

Under the current climatic conditions, establishment outdoors in Germany is not expected. In tropical greenhouses however, establishment is possible if the vector (*Euwallacea* sp.) is present. This is currently the case in various greenhouses – eradication measures are underway (EPPO 2025b).

In areas with suitable climates (see above) and existing host plants, establishment is possible if the vector (*Euwallacea* sp.) is present. This currently appears to be the case in Spain: *Euwallacea fornicatus* sensu lato was detected on *Acer negundo* in 2022 and on *Parkinsonia* sp. in 2023 in a park in Andalusia, southern Spain. This is the first evidence of an established population of the beetle outdoors in Europe. In 2024, further samples of plants (*Acer negundo*, *Persea americana*, and *Ricinus communis*) suspected of being infested with *E. fornicatus* sensu lato were examined. The beetle was detected in all samples (Goldarazena et al. 2025; EPPO 2025c).

### Expected impacts in Germany and the EU

The impact potential of *G. euwallaceae* is not yet sufficiently known. However, damage to avocados, for example, are deemed possible in southern EU member states. Since the fungus has also been found in maples on various continents, maple species in general could also be at risk. The same could apply to plane (*Platanus*) trees, as the fungus has also been found on California sycamore.

### Control of the fungus

Control of *G. euwallaceae* is only possible by eradicating the vector. In the specific case of the tropical greenhouse, where the fungus was found, a ban is in place on removing woody plants and palm trees from the facility. If plants must be destroyed, they are packaged directly in the tropical greenhouse and transported in closed containers for incineration. The entrances from the tropical house to the outdoor areas of the associated recreational facility are closed from April 1 to September 30.

### Conclusions of the PRA

According to the results of the risk analysis being conducted in this study, most probably, *G. euwallaceae* cannot establish outdoors in Germany due to unsuitable climatic conditions. However, it is assumed that *G. euwallaceae* could (further) establish in tropical greenhouses in Germany and other Member States as well as outdoors in southern EU Member States, and may cause significant damage. Therefore, measures should be taken to prevent introduction and spread of this potential quarantine pest and its vectors in accordance with Article 29 of Regulation (EU) 2016/2031 (EU 2016).

In the present case (occurrence in a tropical greenhouse), it is deemed sufficient to contain the infestation inside the tropical greenhouse and not to relocate any infested plants, as the climatic conditions in the areas of origin of the vector *Euwallacea* sp. and other infested areas are not comparable to those in the specific area of Germany. Thus, the risk of establishment in the field is currently considered very low. Removing all host plants in the tropical greenhouse would therefore be disproportionate.

## Discussion

Successful diagnosis of pathogenic fungi depends on many factors starting with the correct selection and disinfection of symptomatic material. During the isolation process on agar plates often a mixture of fungi is growing on woody material and thus the accurate isolation and clean cultivation of the selected pathogenic fungi is the requirement for morphological identification and successful DNA extraction and sequence analyses.

Molecular diagnosis critically depends on the quality of database entries, including both DNA sequence data and accurate species identification. However, well-curated DNA barcode databases require prior expertise in taxonomy and morphology to ensure reliable content. Users of these databases should therefore be aware that some entries may contain errors and interpret results with appropriate caution. Ideally, DNA-based identification should be supported not only by sequence information but also by complementary data and expertise. As discussed in this paper, accurate species-level identification also requires a robust phylogenetic framework. Given the frequent occurrence of taxonomic ambiguities, including synonymy and misidentification, interpretation of molecular diagnostic results should be performed by experts trained in phylogenetics and taxonomy.

PRA is based on accurate diagnosis of the species of concern. In cases where this is not possible, the outcome of the PRA remains vague and may have a high uncertainty, because even if a species belongs to the same genus as a species already found to be harmful to plants, it does not necessarily also to be harmful. Therefore, the categorisation of the organism as a (potential) quarantine pest relies on unambiguous determination of the species (or in rare cases of the subspecies or strain).

Furthermore, categorising the species also depends on the magnitude and likelihood of its risks to plants. In this specific case, the potential of *G. euwallaceae* to cause damage has not yet been sufficiently clarified. Though the phytosanitary risk for Germany is considered low, for southern EU Member States, a phytosanitary risk may exist for e.g. avocado and maple. *Graphium euwallaceae* is primarily a saprophytic fungus and has previously been reported as a pathogen only in sporadic cases. Lynch et al. (2016) consider *G. euwallaceae* as a confirmed pathogen of avocado and ash-leaved maple. Freeman et al. (2019), however, believe that the fungus does not act as a pathogen when naturally infesting the xylem. In the trees infested with *Euwallacea* sp., the associated fungi are not systemic pathogens, but rather weak pseudo-pathogens causing only local lesions (Freeman et al. 2019; Na et al. 2018).

However, as long as no further results are available, because the fungus is associated with *Euwallacea* sp. and other fungal symbionts (e.g. *Fusarium euwallaceae*), it should be categorised in the same way as *F. euwallaceae*, which is regulated as a Union quarantine pest (EU 2016, 2019).

To reduce uncertainty and to gain more clarity in this regard of the outcome of the PRA, pathogenicity tests should be conducted. This is in particular important, when plant health regulations are based on the results of a PRA. If the tests reveal that *G. euwallaceae* is not pathogenic per se, the classification as “potentially quarantine-relevant” should be revised.

**Acknowledgements** T. Pfannenstill (LELF, Plant Health Control) is gratefully acknowledged for providing the sample. We thank C. Tielesch (LELF) for laboratory assistance.

**Author contributions** All authors contributed to the study conception and design as well as to the manuscript. Diagnostics were performed by Katja Boldt-Burisch and Clovis-Douanla-Meli and revised by Björn Hoppe. Pest risk analysis was conducted by Gritta Schrader and revised by all other authors. The first draft of the manuscript was written by Gritta Schrader. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Funding** Open Access funding enabled and organized by Projekt DEAL. Open Access funding enabled and organized by the Project DEAL.

## Declarations

**Conflict of interest** The authors declare that they have no conflicts of interest, financial or non-financial, directly or indirectly related to the work submitted for publication.

## References

Bangash NK, Lee HB (2025) A new species and a new record of *Graphium* from freshwater environment in Korea. Mycobiology 53(3):321–330. https://doi.org/10.1080/12298093.2025.2450892

BfN (2023a) *Acer negundo* L. (Eschen-Ahorn). FloraWeb, Bundesamt für Naturschutz (BfN). Available online: https://www.floraweb.de/php/artenhome.php?suchnr=8. Accessed 14 Apr 2025

BFN (2023b) *Ricinus communis* L. (Rizinus). FloraWeb, Bundesamt für Naturschutz (BfN). Available online: https://www.floraweb.de/php/artenhome.php?name-use-id=6963. Accessed 14 Apr 2025

Bierman A, Roets F, Terblanche JS (2022) Population structure of the invasive ambrosia beetle, *Euwallacea fornicatus*, indicates multiple introductions into South Africa. Biol Invasions 24(8):2301–2312. https://doi.org/10.1007/s10530-022-02801-x

Carrillo D, Duncan RE, Ploetz JN, Campbell AF, Ploetz r, Pena JE (2014) Lateral transfer of a phytopathogenic symbiont among native and exotic ambrosia beetles. Plant Pathol 63(1):54–62. https://doi.org/10.1111/ppa.12073

Carrillo D, Cruz LF, Kendra PE et al (2016) Distribution, pest status and fungal associates of *Euwallacea nr. fornicatus* in Florida Avocado Groves. Insects 7:55. https://doi.org/10.3390/insects7040055

Cruz LF, Menocal O, Dunlap C, Cooperband MF, Cloonan KR, Tabanca N, Caririllo D (2025) Insights on the new symbiotic associations of the tea shot hole borer (Coleoptera: Curculionidae). Front Microbiol. https://doi.org/10.3389/fmicb.2025.1589710

Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM, Gascuel O (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res 36(Web Server issue):W465-9. https://doi.org/10.1093/nar/gkn180

Dzurenko M, Hulcr J (2022) Ambrosia beetles: quick guide. Curr Biol 32(2):R61–R62. https://doi.org/10.1016/j.cub.2021.11.043

EPPO (2025a) *Fusarium euwallaceae*. EPPO datasheets on pests recommended for regulation. https://gd.eppo.int (Accessed 2025 Aug 25)

EPPO (2025b) *Euwallacea fornicatus* sensu lato (XYLBFO). Distribution details in Germany. EPPO Global Database. Available online: https://gd.eppo.int/taxon/XYLBFO/distribution/DE. Accessed 15 Apr 2025

EPPO (2025c) Update on the situation of *Euwallacea fornicatus* sensu lato in Spain. EPPO Reporting Service no. 01–2025, 2025/010. Available online: https://gd.eppo.int/reporting/article-8021. Accessed 14 Apr 2025

EU, Commission Implementing Regulation (EU) (2019) 2019/2072 of 28 November 2019 establishing uniform conditions for the implementation of Regulation (EU) 2016/2031 of the European Parliament and the Council, as regards protective measures against pests of plants, and repealing Commission Regulation (EC) No 690/2008 and amending Commission Implementing Regulation (EU) 2018/2019. https://eur-lex.europa.eu/legal-content/EN/AUTO/?uri=CELEX. Latest consolidation: :02019R2072-20250426 Accessed on: 29 Aug 2025.

EU (2016) Regulation (EU) 2016/2031 of the European Parliament of the Council of 26 October 2016 on protective measures against pests of plants, amending Regulations (EU) No 228/2013, (EU) No 652/2014 and (EU) No 1143/2014 of the European Parliament and of the Council and repealing Council Directives 69/464/EEC, 74/647/EEC, 93/85/EEC, 98/57/EC, 2000/29/EC, 2006/91/EC and 2007/33/EC. Available online: https://eur-lex.europa.eu/eli/reg/2016/2031/oj. Gives access to this document through its ELI URI. Accessed 19 Aug 2025

Freeman S, Sharon M, Dori-Bachash M, Maymon M, Belausov E et al (2016) Symbiotic association of three fungal species throughout the life cycle of the ambrosia beetle *Euwallacea* nr. *fornicatus*. Symbiosis 68(1):115–128. https://doi.org/10.1007/s13199-015-0356-9

Freeman S, Miller G, Protasov A, Maymon M, Elazar M, David-Schwartz R et al (2019) Aposymbiotic interactions of three ambrosia beetle fungi with avocado trees. Fungal Ecol 39:117–130. https://doi.org/10.1016/j.funeco.2018.11.007

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

Goldarenza A, Alcazar-Alba MD, Hulcr J, Johnson AJ (2025) First record of *Euwallacea fornicatus* Eichhoff (Coleoptera: Curculionidae: Scolytinae) in Spain. EPPO Bull 55:146–150. https://doi.org/10.1111/epp.13064

Hulcr J, Black A, Prior K, Chen CY, Li HF (2017) Studies of ambrosia beetles (Coleoptera: Curculionidae) in their native ranges help predict invasion impact. Fla Entomol 100(2):257–261. https://doi.org/10.1653/024.100.0219

Hulcr J, Skelton J (2023) Ambrosia beetles. In Forest entomology and pathology: Band 1: Entomology (Seiten 339–360). Springer International Publishing. https://doi.org/10.1007/978-3-031-11553-0

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform 20:1160–1166. https://doi.org/10.1093/bib/bbx108

Köppen W (1918) Klassifikation der Klimate. Petermann's geographische Mitteilungen. September/Oktoberheft, pp 193–203

Kottek M, Grieser J, Beck C, Rudolf B, Rubel F (2006) World map of the Köppen-Geiger climate classification updated. Meteorol Z 15(3):259–263. https://doi.org/10.1127/0941-2948/2006/0130

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

Li J, Gao X, Liu K, Chen M, Ran Y, Liu C, Lin T, Yin M (2024) Two new species of *Graphium* (Microascales, Ascomycota) associated with pine-infesting bark beetles in China. bioRxiv. https://doi.org/10.1101/2024.09.14.613081

Lynch SC, Twizeyimana M, Mayorquin NJS, Wang DH, Na F, Kayim M et al (2016) Identification, pathogenicity and abundance of *Paracremonium pembeum* sp. nov. and *Graphium euwallaceae* sp. nov.—two newly discovered mycangial associates of the polyphagous shot hole borer (*Euwallacea* sp.) in California. Mycologia 108(2):313–329. https://doi.org/10.3852/15-063

Macleod A, Korycinska A (2019) Detailing Köppen–Geiger climate zones at sub-national to continental scale: a resource for pest risk analysis. EPPO Bull 49(1):73–82. https://doi.org/10.1111/epp.12549

Mendel Z, Protasov A, Maoz Y, Maymon M, Miller G, Elazar M, Freeman S (2017) The role of *Euwallacea* nr. *fornicatus* (Coleoptera: Scolytinae) in the wilt syndrome of avocado trees in Israel. Phytoparasitica 45:341–359. https://doi.org/10.1007/s12600-017-0598-6

Na F, Carrillo JD, Mayorquin JS, Ndinga-Muniania C, Stajich JE, Stouthamer R et al (2018) Two novel fungal symbionts *Fusarium kuroshium* sp. nov. and *Graphium kuroshium* sp. nov. of Kuroshio shot hole borer (*Euwallacea* sp. nr. fornicatus) cause Fusarium dieback on woody host species in California. Plant Dis 102(6):1154–1164. https://doi.org/10.1094/PDIS-07-17-1042-RE

Okada G, Seifert KA, Takematsu A, Yamaoka Y, Miyazaki S, Tubaki K (1998) A molecular phylogenetic reappraisal of the *Graphium* complex based on 18S rDNA sequences. Can J Bot 76:1495–1506. https://doi.org/10.1139/b98-089

Okada G, Jacobs K, Kirisits T, Louis-Seize GW, Seifert KA, Sugita T, Takematsu A, Wingfield MJ (2000) Epitypification of *Graphium penicillioides* Corda, with comments on the phylogeny and taxonomy of *Graphium*-like synnematous fungi. Stud Mycol 45:169–188

Osborn RK, Castro J, Duong TA, Hulc J, Li Y, Martínez M, Cognato AI (2023) Symbiotic fungi associated with xyleborine ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) and the imperative of global collaboration. Ann Entomol Soc Am 116(1):51–71. https://doi.org/10.1093/aesa/saac024

Rambaut A (2012) FigTree version 1.4.0. Computer program and documentation distributed by the author

Schrader G, Kehlenbeck H, Wilstermann A, Kaminski K (2024) Pest risk analysis in Germany over the course of time. J Cultivated Plants/Journal für Kulturpflanzen. https://doi.org/10.5073/JfK.2024.02.03

Schuler H, Witkowski R, Van de Vossenberg B, Hoppe B, Mittelbach M, Bukovinszki T et al (2023) Recent invasion and eradication of two members of the *Euwallacea fornicatus* species complex (Coleoptera: Curculionidae: Scolytinae) from tropical greenhouses in Europe. Biol Invasions 25(2):299–307. https://doi.org/10.1007/s10530-022-02929-w

Six DL (2012) Ecological and evolutionary determinants of bark beetle—fungus symbioses. Insects 3(1):339–366

Swofford DL (2003) PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version 4. Sinauer Associates, Sunderland. https://doi.org/10.1111/j.0014-3820.2002.tb00191.x

Trollip C, Carnegie A (2025) First record of *Graphium* species associated with *Euwallacea perbrevis* in Australia. APS Online Publications. https://doi.org/10.1094/PDIS-02-25-0319-PDN

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172:4238–4246

Bierman A, Roets F, Terblanche JS (2023) Correction:Population structure of the invasive ambrosia beetle, Euwallacea fornicatus,indicates multiple introductions into South Africa. Biol Invasions, 25(2):635-636. https://doi.org/10.1007/s10530-022-02916-1

IPPC (2001)International Standard on Phytosanitary Measures (ISPM) 11. Pest Risk Analysisfor Quarantine Pests. IPPC, FAO, Rome. International Plant ProtectionConvention, Rome

White T,Bruns T, Lee S, Taylor J (1990) Amplification and Direct Sequencing of Fungal RibosomalRNA Genes for Phylogenetics. Academic Press, New York.

Jacobs K,Bergdahl DR, Wingfield MJ, Halk S, Seifert KA, Bright DE, Wingfield BD (2004) Leptographiumwingfieldii introduced into North America and found associated with exotic *Tomicuspiniperda* and native bark beetles. Mycological Research 108: 411–418.

Mendel Z, Protasov A, Sharon M, Zveibil AYSB,Yehuda SB, O'Donnell K, RabagliaR, Wysoki M, Freeman S (2012) An Asian ambrosia beetleEuwallacea fornicatus and its novel symbiotic fungus Fusarium sp. pose aserious threat to the Israeli avocado industry. Phytoparasitica, 40(3),235-238.

EPPO (2024)EPPO Standards – PM 5 Pest Risk Analysis. Available online: https://www.eppo.int/RESOURCES/eppo_standards/pm5_pra. Accessed 7 Apr 2026.