INVASION NOTE



Recent invasion and eradication of two members of the *Euwallacea fornicatus* species complex (Coleoptera: Curculionidae: Scolytinae) from tropical greenhouses in Europe

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Abstract Ambrosia beetles of the *Euwallacea fornicatus* species complex are emerging tree pests with a broad host range including important agricultural crops. Native to Southeast Asia, these species were introduced into various countries, where they cause considerable damage to many tree species. Here we report several outbreaks of *E. fornicatus s.l.* in

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Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for National and International Plant Health, Braunschweig, Germany Europe. The first individuals were found in 2017 in a palm house of a botanical garden in Poznan (Poland) whereas in 2020 an outbreak was detected in a tropical greenhouse in Merano (Italy). In 2021, two additional outbreaks were detected in two greenhouses in Germany, in Erfurt and Berlin. For both cases in Germany it was possible to trace back the invasion to a distributor of exotic plants in the Netherlands where several infested plants were detected. Molecular analyses show that individuals from Poland and Italy are

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A. Battisti DAFNAE, University of Padova, Padua, Italy genetically identical but belong to a different mitochondrial clade than individuals in Germany which are identical to most individuals of two greenhouses in the Netherlands. Moreover, in the two greenhouses in the Netherlands we found beetles that belong to another haplotype of *E. fornicatus* and two haplotypes of *E. perbrevis*, a species in the *E. fornicatus* complex, which has not been previously intercepted in Europe. Our study provides novel insights into the invasion history of *E. fornicatus* and the eradication measures in Europe. Considering the potential of introduction and establishment of *Euwallacea* ambrosia beetles, particular attention should be paid to monitor the presence of these pests in tropical greenhouses across Europe.

Keywords Ambrosia beetle · *Euwallacea* fornicatus · *Euwallacea perbrevis* · Invasive species · Polyphagous shot hole borer · Tea shot hole borer

Introduction

Members of the Euwallacea fornicatus species complex (Coleoptera: Curculionidae: Scolytinae) are agricultural pests in most parts of the world (Beaver 1976; Li et al. 2016). They are one of a few examples of ambrosia beetles that are able to infest healthy plants (Gomez et al. 2019). Based on a recent reclassification, the E. fornicatus species complex is comprised by four nearly identical species including the Polyphagous shot hole borer (PSHB) E. fornicatus, the Tea shot hole borer (TSHB) E. perbrevis, the Kuroshio shot hole borer (KSHB) E. kuroshio, and E. fornicatior (Smith et al. 2019). Species of the E. fornicatus species complex have a broad host range of 412 plant species in 75 families (Gomez et al. 2019) including important agricultural crops such as tea (Camellia sinensis), mango (Mangifera indica) and avocado (Persea americana) (Eskalen et al. 2013; Carrillo et al. 2016); Danthanarayana 1968; Yamaguchi et al. 2006). They are associated with several symbiotic fungi including Fusarium euwallaceae, Graphium euwallaceae and Paracremonium pembeum (syn. Acremonium pembeum) (Carrillo et al. 2016; Freeman et al. 2016; Lynch et al. 2016) causing pseudo-pathogenic interaction (i.e. where the pathogenic effect only occurs with the activity of the beetle) with the xylem of their hosts (Eskalen et al. 2012, 2013; Freeman et al. 2013, 2019).

Euwallacea fornicatus s.l. is native to Southeast Asia with confirmed records in China, Japan, Malaysia, the Philippines, Taiwan, Sri Lanka, Thailand, and Vietnam (CABI 2021; Stouthamer et al. 2017). Outside its native range, E. fornicatus s.l. invaded various countries in North and Central America (USA, Panama, Costa Rica), Africa (South Africa, Réunion), Oceania (Australia, Papua New Guinea, Fiji and other countries), and Israel (Kirkendall and Ødegaard 2007; Cooperband et al. 2016; CABI 2021). In Europe, E. fornicatus s.l. is considered a quarantine pest and is included in the Annex II Part A (Commission Implementing Regulation EU 2019/2072). Its wide host range makes E. fornicatus s.l. a serious threat for agriculture but also forestry, ornamental plants, and botanical gardens.

Several outbreaks were recently discovered in tropical greenhouses of botanical gardens in Europe. In 2017, specimens of E. fornicatus were found in a palm house in Poznań, Poland (EPPO 2019), whereas various tropical plants have been attacked by E. fornicatus in a greenhouse in Merano, Italy (EPPO 2020). More recently, two other cases have been reported in Germany in two tropical greenhouses in Thuringia (EPPO 2021a) and Berlin (EPPO 2021b). Trace back investigations linked the finding in Berlin to a greenhouse in the Netherlands where several larvae and adults of E. fornicatus s.l. were detected (EPPO 2021c). Although tracing information of consignments has been used to establish the origin of infested plants, analysis of molecular data may provide direct insights into the invasion history of Euwallacea and thus into the dynamics of introduction events across the different locations. Here we perform a comparative genetic analysis of insects from different locations in Europe and attempt to trace back the introduction pathway of the beetles. Moreover, we report the subsequent eradication measures. Our study provides new insights into the introduction and the subsequent steps to eradicate these invasive insect pest species.

Materials and methods

Detection and identification

In Poland, in March 2017, a sacred fig tree (Ficus religiosa) in the Poznań Palm House showed branch dieback and visible frass, typical symptoms for infestation with ambrosia beetles. An approximately 1 m long branch of the tree was cut and placed in a breeding container. Emerging beetles were collected for taxonomic identification. In Italy, in April 2020, several trees in the tropical greenhouse of the Gardens of Trauttmansdorff Castle in Merano, showed boreholes and ejection of wooden debris. Emerging beetles were directly collected from infested plants, from the walls of the greenhouse and extracted from unbaited trap logs of Acer negundo that were deployed in the greenhouse. In Germany, in January 2021, two plants, one Mangifera indica and one Tectona grandis, showed symptoms of a wood boring beetle infection in a tropical greenhouse in Erfurt. Two months later several plants in a greenhouse in Berlin showed resin flows on stems and boreholes with small protruding tubes of compacted sawdust. In the Netherlands, in 2021, several plants were found to be infested at two plant nurseries belonging to the same company in Westland, South-Holland (later referred to as NL1 and NL2). A subset of emerging beetles and beetles extracted from the plants were collected and stored in absolute ethanol for molecular analysis.

Identification of the insect material was carried out using taxonomic keys available in the literature (Smith et al. 2019) by the Poznań University of Life Sciences in Poland, the Laboratory for Virology and Diagnostics of the Research Centre Laimburg in Italy, the state office for plant protection in Thuringia, the federal plant protection agency Berlin, the National reference laboratory of the Julius-Kühn Institute (JKI) in Braunschweig in Germany and by the NIVIP of the NVWA in the Netherlands.

Eradication and surveillance

In Poland, the whole sacred fig tree was covered with an insect-proof net (Storanet® BASF, Germany) impregnated with alpha-cypermethrin immediately after the appearance was noticed. The tree was subsequently removed and burnt under controlled conditions. The soil around the roots was excavated and the surrounding area was treated with the fungicide Topsin M (Thiophanate-Methyl, 100 ppm). Beetle surveillance with 4 triangle barrier traps IBL-2 (Chemipan, Warsaw, Poland) baited with 98% ethanol started immediately and continued for a year after removal of the infested tree. Furthermore, plant health conditions in the palm house were monitored by visual observation of symptoms.

In Italy, an intense monitoring has started inside the greenhouse and in the surrounding area after the first detection of the ambrosia beetles. Due to the advanced outbreak, all the plants in the greenhouse, including their roots, were removed and destroyed under official control in June 2020. The greenhouse was also subjected to solarization for a period of three months. Simultaneously, three sticky traps baited with quercivorol and alpha-copaene according to Kendra et al. (2019) and several trap logs of Acer negundo (diameter 4-10 cm, length 30-60 cm) were placed inside and outside the greenhouse to verify the possible presence of adults. In the area external to the greenhouse, two sticky traps baited with quercivorol and alpha-copaene were also deployed at 500 and 1,000 m from the tropical greenhouse in each cardinal direction, for a total of eight traps. All traps were checked weekly. Known host plants of the beetle (Acer spp., Citrus spp., Platanus spp., etc.) were monitored for boreholes especially after favorable weather conditions.

In Erfurt, the infested Tectona grandis was removed and destroyed whereas only infested branches of the Mangifera indica were eliminated. Known host plants were visually inspected for bore holes weekly from March until December 2021. Additionally, monitoring inside and around the greenhouse was performed using multi-funnel traps (Witasek, Austria) baited with quercivorol. All traps were checked weekly. In Berlin only symptomatic parts of the trees and shrubs were removed and destroyed since the plants are needed as shelter plants for megabats which are kept inside the greenhouse. The inner greenhouse was declared as infection zone, and visual inspections for symptoms on potential host trees and shrubs were conducted biweekly. In addition, multi-funnel traps baited with quercivorol were installed and monitored in the inner greenhouse as well as in the surrounding parts of the building. Moreover, freshly cut trap logs of Acer negundo were placed inside the greenhouse, outer parts of the building-complex (buffer zone), as well as in the surrounding parts of the building (monitoring-zone). Traps and trap logs were controlled biweekly.

In the Netherlands, the sale and transport of all woody plants and palms in the two greenhouses was put on hold. In the nursery NL1, only infested plants and some plants with ambiguous symptoms were removed and treated. The glasshouse was then monitored using eight cross-vane traps (Crosstrap®, Econex, Spain) and four white sticky traps baited with quercivorol and alpha-copaene (Kendra et al. 2019), and subsequently for 17 weeks using 16 white sticky traps. In the nursery NL2, all plants were removed. The glasshouse was then thoroughly cleaned and kept free of plants for eleven days. During that period the glasshouse was also monitored using 12 cross-vane traps and 12 white sticky traps baited with quercivorol and alpha-copaene.

Molecular identification

A total of 64 individuals were analyzed including three from Poland, nine from Italy, 20 from Germany (three from Erfurt and 17 from Berlin) and 32 from the Dutch outbreak locations (18 from NL1 and 14 from NL2). Individuals were genotyped using the primers Lep-F1/Lep-R1 (Hebert et al. 2004). Details about the extraction protocol, PCR conditions and sequencing can be found in supplementary document S1.

Relationship of the obtained sequences was determined using partial mitochondrial COI sequences obtained from NCBI GenBank and the 64 partial COI sequences generated in this study. Overall, 74 sequences were selected ensuring representation of the known haplotypes in the PSHB, KSHB clades and the two subclades TSHBa and TSHBb. When identical haplotypes were obtained for samples sequenced in this study, a single representative was included in the clustering analysis. Sequences were aligned using the MAFFT aligner (Katoh et al. 2002; Katoh and Standley 2013) incorporated in Geneious Prime v2021.1.1 (BioMatters, New Zealand). Terminal positions in the alignment that were not covered by all sequences in the dataset were masked resulting in a 564 bp alignment used for clustering analysis. A maximum likelihood tree was constructed with FastTree (Price et al. 2010) using the generalized time-reversible (GTR) model and 1,000 bootstraps to determine confidence levels of internal nodes. The COI sequence of *Euwallacea andamanensis* isolate PR13-238 (KU727039) was used to root the *E. fornicatus s.l.* sequences.

Results

Detection and eradication

Taxonomic identification revealed that all specimens from the different localities are *E. fornicatus s.l.* The identification on a species level was assessed by genotyping a subset of samples using a DNA barcoding approach (see results below).

In Poland, the sacred fig sample yielded over 1,000 specimens of *E. fornicatus*. No beetles were caught by the traps for one year after the tree was removed. Plants occurring in the same pavilion as the infested sacred fig and plants in neighboring pavilions, which were subjected to regular inspections, did not show symptoms of ambrosia beetles and fungal infection.

In Italy, a total of 28 trees of 21 different species (Annona muricata, Artocarpus heterophyllus, Averrhoa carambola, Bixa orellana, Bulnesia arborea, Cananga odorata, Clausena lansium, Crescentia cujete, Debregeasia edulis, Dimocarpus longan, Ficus altissima, Ficus sp., Justicia sp., Kigelia africana, Melicoccus bijugatus, Mangolia champaca, Millettia brandisiana, Persea americana, Terminalia catappa, Terminalia buceras, Theobroma cacao) showed boreholes and ejection of wooden debris. Intensity of infestation, diameter of infested trees and distribution of the boreholes on the plant differ strongly between species and individual plants. A. muricata and B. orellana were the most heavily infested plants. The highest density of boreholes was observed next to fresh and older cut branches and on thinner parts in the crown/upper stem. The presence of boreholes was also observed in twigs less than 2 cm of diameter of Dimocarpus longan and Justicia sp. trees. Other plants grown in the greenhouse such as Bombax ceiba, Carica papaya, Caryota urens, Casimira edulis, Coffea arabica, Crescentia alata, Cyathea australis, Diospyros digna, Garcinia livingstonei, Manilkara zapota, Pandanus utilis, Pimenta dioica, Saraca indica, Syzygium cumini, and Syzygium jambos, which are not described as host plant of *E. fornicatus*, were not infested. No beetles were caught in the traps deployed outside of the tropical greenhouse and visual inspection of susceptible host trees did not result in finding of symptoms of beetle attack. The last beetles were caught on the 29th of May 2020.

The two recent findings in Germany revealed that in Erfurt just two plants, one *Mangifera indica* and one *Tectona grandis*, were attacked by *E. fornicatus*, whereas in Berlin 136 shrubs and trees of *Clusia rosea*, *Heteropanax* sp., *Ficus* sp., and *Mangifera indica* showed symptoms. After the eradication measures no symptomatic trees were observed, and no beetles were caught after the 8th of March 2021 in Erfurt. In Berlin the eradication is still in process as at present (September 2022) beetles are still being caught.

Following a trace-back of the notification in the Erfurt case, we were able to link these infestations to a Dutch greenhouse for commercial sale of tropical plants in South-Holland (NL1). A follow up survey highlighted that a total of 12 plants of Ficus microcarpa, Bauhinia x blakeana, F. microcarpa 'Panda', Ficus sp., Ficus maclellandii 'Alii' and Ficus 'Amstel King' were infested. Moreover, a survey in another greenhouse associated with the company (NL2) resulted in the discovery of 15 additional symptomatic trees of F. benjamina 'Exotica', F. foliole and Ficus lyrata. At the greenhouse NL2 after removing the plants and cleaning the greenhouse no beetles were caught. Therefore, in August 2021 Euwallacea s.l. was declared to be eradicated. The eradication of E. fornicatus at location NL1 has been successfully concluded in February 2022 when no beetles were caught over 12 weeks.

Reconstruction of the invasion

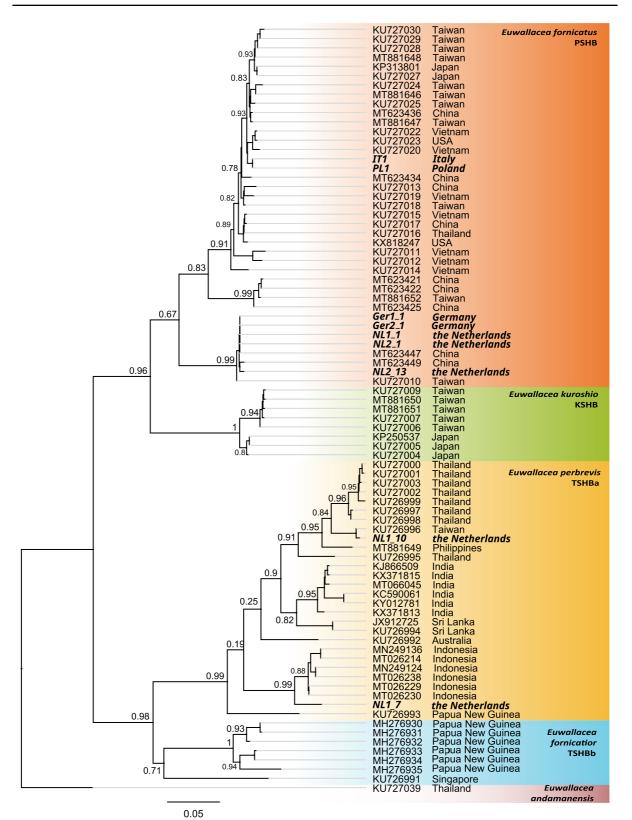
Trace-back investigations revealed that the *F. religiosa* tree infested with *E. fornicatus* in Poland was imported in November 2016 from the Netherlands. However, it was not possible to trace it back to the exact vender. Since no other tree showed signs of infections in Poland, we assume that this tree was already infested with *E. fornicatus* when it was planted in the greenhouse in Poznan. The first symptoms were observed in March 2017 and therefore the infection has already been detected four months after its introduction. The advanced outbreak in Italy with

28 different trees attacked at the time of detection, hindered a detailed reconstruction of the invasion. The greenhouse in Italy was established in 2014 and most plants were purchased between 2013 and 2014. No symptoms of beetle attack were reported in the first years following the establishment. Additionally, one T. cacao plant was replaced in 2018, which was purchased in the Netherlands. Two years later this plant was discovered to be attacked by E. fornicatus. We therefore assume that this plant was the source of the E. fornicatus outbreak in Italy. Since the symptoms where observed the first time in April 2020, the beetles were able to establish and subsequently attacked other plants in the greenhouse within two years. Both infested plants in Erfurt, and most of the infested plants in Berlin were acquired and imported from a distributor of exotic plants in the Netherlands in 2020. In both cases the symptoms were detected a few months later which allowed to trace the infection back to a specific vender.

Due to the advanced outbreak in the two greenhouses in the Netherlands it was not possible to trace back the reconstruction of the introduction. The company has been importing plants from various countries including Malaysia, Thailand, Costa Rica and China over the last years and thus the outbreak may have multiple origins.

Molecular identification

All individuals from Poland and Italy were genetically identical (Genbank acc. number ON037479) but different from the individuals found in Germany and the Netherlands. The haplotype described in Poland and Italy clustered with haplotypes of the PSHB clade which according to Smith et al. (2019) is E. fornicatus. However, none of the previously published haplotypes were identical to the haplotype described in these two localities. The cluster analysis showed that this haplotype was most related to a haplotype present in Vietnam (Stouthamer et al. 2017; Fig. 1). In contrast, all individuals from Germany share the same haplotype (Genbank acc. number ON037480) which was identical to a haplotype described in China and clustered with E. fornicatus in the PSHB clade 3A (Fig. 1). The same haplotype was described in the Netherlands in 13 individuals of location NL1 and in all 12 individuals from location NL2.



◄Fig. 1 Phylogenetic tree based on partial (564 bp) mitochondrial *cox1* gene sequences representing the relation of the individuals found in Europe (in bold and italics) with haplotypes described in other studies

Moreover, two additional individuals from the Netherlands (NL2) had a different haplotype (Genbank acc. number ON037481) which was closely related to the predominant haplotype in the two populations in Germany and the Netherlands, differentiated by two single nucleotide positions (Fig. 1). Additionally, five individuals from NL1 belonged to two different clusters within the TSHBa clade (Genbank acc. ON037482 and ON037483) which according to Smith et al. (2019) is now considered *Euwallacea perbrevis*. The haplotype of three individuals clustered with haplotypes previously described in Indonesia whereas two individuals clustered with a haplotype described in Taiwan (Fig. 1).

Discussion

Botanical gardens and greenhouses are hotspots for invasive species (Wang et al. 2015). The deliberate import of alien plants might lead to the invasion of non-native plants (Hulme 2011) but might also lead to the introduction of associated insects (Scott-Brown et al. 2018). Here we show an additional example of a recent introduction of two non-native insect pests in botanical gardens of Europe and trace back their invasion route and document the eradication measures.

Ambrosia beetles of the Euwallacea fornicatus species complex are invasive species introduced into various continents but had not been detected in Europe. Here we describe the recent outbreaks of E. fornicatus in four tropical greenhouses in Poland (Poznan-2019), Italy (Merano-2020), and Germany (Berlin and Erfurt-2021). We also reconstructed the invasion history and traced back part of its invasion route to two greenhouses of a commercial distributor of exotic plants in the Netherlands (Westland-2021). Molecular characterization showed that all individuals from Poland and Italy share the same mitochondrial haplotype, suggesting that they were likely introduced from the same source population. The haplotype belongs to the Polyphagous shot hole borer clade (Stouthamer et al. 2017) and is most related to a haplotype found in Vietnam (Stouthamer et al. 2017; Fig. 1). Since the haplotype was not described elsewhere, we could not determine the exact source population. In contrast, the populations in Germany and the Netherlands were identical to a haplotype recently described in China, suggesting a direct introduction pathway between Asia and the Netherlands. While in the former cases we were not able to trace back the introduction, we assume that both cases originated from the same source population. The genetic identity of individuals collected in both glasshouses in Germany confirmed that these cases were introduced via one of the greenhouses of the commercial nursery in the Netherlands. The additional detection of E. perbrevis in a greenhouse in the Netherlands highlights at least one additional introduction event of a species which has not been found elsewhere in Europe. The detection of two distinct haplotypes of E. perbrevis, which clustered with haplotypes from two different geographic regions in Asia, indicates that E. perbrevis might have been introduced by two independent introduction events. Since E. fornicatus and E. perbrevis coexist in many Asian countries (Stouthamer et al. 2017) we cannot exclude that they have been introduced with a tree infested with both species.

The outbreaks in the different localities were in different epidemiological phases: E. fornicatus in Poland was present in a single sacred fig tree (Ficus religiosa) and has not been found in any other tree species in the same greenhouse. It seems likely that the E. fornicatus was introduced to Poland with an infested F. religiosa tree in 2016 and eradicated a few months later in the initial phase of its establishment. In contrast, the outbreak in Italy was more advanced when it was discovered in 2020 when beetles were detected already in 28 different plants belonging to 21 species. The most plausible explanation is that the ambrosia beetle was introduced with the T. cacao plant purchased in 2018 and subsequently attacked other plant species in the greenhouse where the outbreak was detected two years later. In Erfurt both infested trees of Mangifera indica and Tectona grandis, as well as most of the 136 infested trees in Berlin were imported from the Netherlands. In both cases, the presence of the beetle was detected a few months after their introduction. In Poland, Italy, Erfurt and the Netherlands, the eradication of the plants (and infected part of the plants) resulted in the successful eradication, whereas the eradication process in Berlin is still in progress.

Due to the constant acquire of exotic plants from various regions it was not possible to reconstruct the introduction route to Europe. However, the finding of several haplotypes from various geographic regions highlight that both *Euwallacea* species were likely introduced by multiple introduction events. All affected greenhouses are surrounded by several native and non-native trees and shrubs, known as potential hosts for *E. fornicatus*. However, in none of the surveys of the different localities beetles outside of the greenhouse were caught. Although *E. fornicatus* might not be able to survive the outdoor conditions prevailing in Poland, Germany and the Netherlands, mediterranean conditions in Italy might allow an establishment of the beetles in this area.

The occurrence of *E. fornicatus* in the greenhouse of a retailer in the Netherlands and especially the detection of *E. perbrevis*, which has not been reported elsewhere in Europe, highlights the need of more awareness of exporters, importers and phytosanitary authorities for this pest, requiring comprehensive examinations of exotic plants prior re-sale. While eradication may be relatively simple in a greenhouse environment, it becomes much more problematic once a population is established outdoors. In order to reduce the likelihood of establishment in nature, we argue that surveillance should be intensified in commercial nurseries and tropical greenhouses throughout Europe.

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Author contributions AB, HS conceived and designed the study. Survey, monitoring and eradication was performed by RW, MB and AM in Poland, SS, AA and AB in Italy, BH, MM, UL and SR in Germany and TB and BvdM in the Netherlands. RW, BH, MM and BvdM performed the taxonomic identification, HS and BvdV performed the molecular data analysis, HS and AB wrote the manuscript with contribution of all authors.

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Availability of data and material Cytochrome oxidase I (COI) sequences are available on the Genbank with the following accession numbers: ON037479-ON037483. The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Ethics approval This article does not contain any studies with human participants or animals (vertebrates) performed by any of the authors.

Consent to participate Not applicable.

Consent for publication Not applicable.

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