Journal of Pest Science
https://doi.org/10.1007/s10340-021-01443-7

ORIGINAL PAPER

A first inference of the phylogeography of the worldwide invader *Xylosandrus compactus*

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Received: 29 June 2021 / Revised: 9 September 2021 / Accepted: 6 October 2021

Abstract

Native to Southeastern Asia, the ambrosia beetle *Xylosandrus compactus* is invasive worldwide. Its invasion is favoured by its cryptic lifestyle, symbiosis with a fungus that facilitates a broad range of host plants, and predominant sib-mating reproduction. *X. compactus* invaded Africa more than a century ago and the Americas and Pacific Islands in the middle of the twentieth century. It was not detected in Europe before 2011, when it was first reported in Italy before quickly spreading to France, Greece and Spain. Despite the negative environmental, agricultural and economic consequences of the invasion of *X. compactus,* its invasion history and main pathways remain poorly documented. We used COI and RAD sequencing to (i) characterise the worldwide genetic structure of the species, (ii) disentangle the origin(s) of the non-native populations on the three invaded continents and (iii) analyse the genetic diversity and pathways within each invaded region. Three mitochondrial lineages were identified in the native range. Populations invading Europe and the American-Pacific region originated from the first lineage and were only slightly genetically differentiated at nuclear SNP markers, suggesting independent introductions from close sources in or near Shanghai, ca. 60 years apart. Populations invading Africa originated from the second lineage, likely from India or Vietnam.

Keywords Bioinvasion · Invasion route · Black twig borer · COI · RAD sequencing · Ambrosia beetle

Key message

- *Xylosandrus compactus* is native to Asia and invasive in Africa, the Americas, and the Pacific Islands, and it recently invaded Europe.
- We used COI and RAD sequencing to identify its source populations and decipher its invasion history.
- Populations from Europe and the American-Pacific region are closely related, show almost no genetic diversity and presumably originated from independent introductions from Eastern China.
- The invasion in Africa likely originated from Southeastern Asia, potentially from India or Vietnam.
- Invasions probably happened through the international trade of living plants.

Communicated by Antonio Biondi.

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Published online: 20 October 2021

Introduction

Biological invasions have been increasing dramatically in the last decades, with no sign of plateauing, and are now one of the main threats to biodiversity and ecosystems health and services (Pejchar and Mooney 2009; Seebens et al. 2017, 2020; Sardain et al. 2019). Invasions are known to cause biodiversity loss (Dueñas et al. 2021), ecosystem disruption (Morales et al. 2017; Simberloff et al. 2013; Kenis et al. 2008), economic loss (Bradshaw et al. 2016) and human health problems (Schindler et al. 2015; Jones 2017; Jones and McDermott 2017). International trade plays a major role in insect biological invasions, dispersing pest species between geographically isolated ecosystems (Gippet et al. 2019). Invasion scenarios can however be diverse, for example resulting from single or multiple introductions from the species' native range, and may or may not involve strong bottlenecks depending on the number of dispersed propagules. One of the invaded regions can also serve as a source for further introductions, a phenomenon known as "bridgehead effect" (Lombaert et al. 2010). Understanding the routes of invasions and determining the source populations is thus critical in developing management strategies to prevent further introductions. The most effective way to retrace invasive species' invasion history is to study their worldwide genetic structure (Estoup and Guillemaud 2010).

Bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) are among the most successful invasive species groups. They represent 0.2% of insect species but more than 50% of insect interceptions in ports of entry (Hulcr and Dunn 2011). Ambrosia beetles take their name from their obligate association with symbiotic ambrosia fungi. The females dig galleries into the xylem of their host plant, where they inoculate spores of their fungal symbiont, which will be used as the only food source by both larvae and adults. This symbiosis allows them to attack a broad range of host plant species and easily shift to new hosts in invaded ranges, which is a major reason for their success as invaders (Kirkendall et al. 2008). Because they are tiny insects living inside galleries most of their life, they can easily travel long distances unspotted, hidden in living plants or wood packaging material (Raffa et al. 2015). While most ambrosia beetles are harmless in their native range, attacking only dead or weakened trees, some species can attack healthy trees and economically important crops in the ecosystems they invade (Hulcr et al. 2017; Kühnholz et al. 2001; Ploetz et al. 2013). Ambrosia beetles can also spread pathogenic fungi and diseases in naive ecosystems (Hulcr and Dunn 2011). For instance, the laurel wilt disease caused by the pathogenic fungus *Raffaela* spread by the redbay ambrosia beetle (*Xyleborus glabratus*) has killed hundreds of millions of trees in the Lauraceae family since it was first detected in the USA in 2002 and is responsible for a $356 million annual loss in the avocado industry (Evans et al. 2010).

Xylosandrus is a particularly successful genus of invading ambrosia beetles. Out of the 54 *Xylosandrus* species presently recognised, four are worldwide invaders causing major losses in plant nurseries and cultivations, namely *X. crassiusculus*, *X. germanus*, *X. morigerus* and *X. compactus* (Gugliuzzo et al. 2021; Dole et al. 2010). Like other ambrosia beetles, *Xylosandrus* species have biological and ecological characteristics favouring invasion (Kirkendall and Odegaard 2007). They are haplodiploid (i.e. non-fertilised eggs give haploid males, while fertilised eggs give diploid females) and predominantly mate between siblings as females usually mate with their brothers in maternal galleries before dispersing. More, as adults are relatively long-lived, a single unmated *Xylosandrus* female is able to establish a population by mating with its haploid male offsprings (Jordal et al. 2001). The combination of haplodiploidy and regular inbreeding allows to lower inbreeding depression by purging deleterious alleles (Peer and Taborsky 2005), which prevents detrimental effects of low population density typical for regularly outcrossing diploid species, such as the mate-finding Allee effect (Gascoigne et al. 2009). The invasive *Xylosandrus* species were reported on hundreds of host species belonging to dozens of plant families (Weber and McPherson 1983; Browne 1961). This could be caused by generalist genotypes able to live in multiple host plants rather than complexes of multiple genotypes specialised on diverse plant families. Indeed, Andersen et al. (2012) showed that deeply diverging genotypes of *X. morigerus* shared broad and completely overlapping niches without any sign of host specialisation.

The genetic structures of populations of these four species have been studied in the last decade, but the worldwide patterns of invasions were only addressed for *X. germanus* (Dzurenko et al. 2020) and *X. crassiusculus* (Storer et al. 2017; Ito and Kajimura 2009; Landi et al. 2017). Despite similar ecological characteristics, these two species were proved to have drastically different invasion histories. In particular, all non-native populations of *X. germanus* proved to originate from a single region in Japan, and invasion in Europe and in North America occurred independently. Results based on a mitochondrial and a nuclear gene further suggested that only one introduction event occurred in Europe, while several introductions were suggested in North America. On the other hand, genetic structure and invasion history of *X. crassiusculus* seem more complex. Cryptic diversity was identified worldwide, and divergent lineages were introduced in different regions of the world, with each invasive population potentially resulting from numerous

introductions, or from a single genetically diverse introduction, or a mix of both.

Very little is known about *Xylosandrus compactus* genetic structure and invasion history. The only genetic study focusing on *X. compactus* so far was centred on India and based on a single mitochondrial marker, showing a very low genetic diversity in spite of a sampling including almost 200 specimens (Kiran et al. 2019). However, this work did not discuss *X. compactus* invasion history. Yet, *X. compactus* is a worldwide invader native to temperate and tropical regions of Asia. It is reported on more than 220 host species from more than 60 families (Ngoan et al. 1976; Beaver et al. 2014), including several plants of economic importance, such as cacao, mango, avocado and coffee (Oliveira et al. 2008). Solely in Uganda, *X. compactus* is responsible for $40 million annual loss due to damage on coffee plantations (Egonyu et al. 2015). It is also known to spread potentially pathogenic fungi such as *Fusarium solani* (Bosso et al. 2012). *X. compactus* is still expanding worldwide and colonising new territories, notably in Europe where it spreads at an alarming rate. It has been present in Madagascar and Africa for more than a century, and it colonised North America in the 1940s, Hawaii in 1964 and South America in the late 1970s. Colonisation of Europe is much more recent, as it was first detected in Italy in 2011 (Garonna et al. 2012), in France in 2014 (Roques et al. 2019), in Greece (Spanou et al. 2019) and Mallorca (Balearic Islands) (Leza et al. 2020) in 2019, continental Spain in 2020 (Gallego et al. 2020) and Corsica in 2021 (A.R., pers. obs.). Species distribution modelling suggests that *X. compactus* could spread to most Mediterranean regions and along the Atlantic coast from Portugal to the United Kingdom. It could also establish in new countries such as Australia, New Zealand or Mexico, where environmental conditions are predicted to be suitable (Urvois et al. 2021).

The aim of the present study was to characterise the global genetic structure and invasion history of *X. compactus* worldwide, with a focus on Europe where it was the most recently introduced. We used both mitochondrial and nuclear markers to test whether they bring consistent information, as these two types of markers can be differently affected by the species' demographic history and could show contrasting patterns (Toews and Brelsford 2012). Moreover, we used genome-wide nuclear data based on Restriction-site Associated DNA (RAD) sequencing (Davey and Blaxter 2010) to describe at high resolution the species' genetic structure. Our objectives were (i) to compare the worldwide genetic structure obtained with mitochondrial and nuclear markers, and to determine if the species consisted of differentiated lineages; (ii) to disentangle the origin(s) of the non-native populations on the three invaded continents (Africa, America and Europe), testing if they were colonised independently and if each continent was invaded once or several times; and (iii) to analyse the genetic diversity and pathways within each invaded region. Answering these questions is timely to develop management strategies and propose plans for efficient epidemiological surveillance and early detection for this highly invasive pest.

Material and methods

Insect sampling

We collected *Xylosandrus compactus* females from 29 locations (Table 1). *X. compactus* is commonly described as native to Southeastern Asia. Still, the exact boundaries of its native range are unknown and it is not possible to know whether it should be considered invasive in the periphery of its Asian distribution, such as India. To simplify, we decided to consider all Asian localities as part of its native area. China was the only country sampled in the presumptive native range of the species. Low sampling coverage in invasive species' native range is a common issue as they often cause less damage in their native area and are thus harder to find there. We collected specimens at 25 sites in the invaded range, in 6 countries distributed on three continents. The insects were obtained either directly from the host tree, from traps baited with ethanol or more specific attractants (Roques et al., in prep) or from collections. Whenever possible, insects from each location were caught from different trees and traps, and at different dates, to minimise within-location inter-individual relatedness. Individuals were stored in 96% ethanol and at -18 °C until DNA extraction.

DNA extraction

Mycangia were removed, and each specimen was washed with 70% alcohol and cleaned with a paintbrush to limit potential fungal contamination. DNA was then extracted from the whole insect using the Macherey–Nagel NucleoSpin Tissue kit following the manufacturer's instructions, with two successive elutions in 50 µL elution buffer BE to increase DNA yield, and stored at -18 °C.

Mitochondrial DNA sequencing

We sequenced between 1 and 8 insects per location for a total of 96 specimens. We amplified the barcode COI fragment via PCR using the primers HCO2198 (5' –TAAACT TCAGGGTGACCAAAAAATCA – 3') and LCO1490 (5' – GGTCAACAAATCATAAAGATATTGG – 3') (Folmer et al. 1994) (Table 1). The PCR was performed as follows: denaturation for 5 min at 94 °C followed by 35 cycles of amplification of 45 s at 94 °C, 50 s at 47 °C and 90 s at 72 °C and finally 5 min at 72 °C. PCR products were cleaned using

Table 1 Summary of the localities sampled and specimens used in the COI and RAD sequencing analyses. UFFE is short for University of Florida's Forest Entomology Laboratory and uffeID represents the sample's unique identifier in the UFFE collection database. PACA is short for the French region Provence-Alpes-Côte d'Azur

Range	Sender (GenBank accession number/uffeID)	Country	State/Province/Region	Locality	Latitude	Longitude	No. in COI analysis (haplotype)	No. in RAD analysis (group)
Africa	GenBank (GU808707.1)	Ghana	Western Region	Ankasa	5.454129	–2.049559	1 (B2)	–
Africa	Fabrice Pinard	Uganda	Central Region	Bunjako Island	0.002465	32.133916	2 (B2)	7 (group 3)
Asia	UFFE (31,708)	China	Fujian	Shuyang	27.159615	119.685488	2 (A5)	2 (group 4)
Asia	UFFE (31,704)	China	Guizhou	Zunyi	27.702703	106.923687	2 (A4)	2 (group 4)
Asia	GenBank (MN620067.1)	China	Hong Kong	–	22.351683	114.167294	1 (C)	–
Asia	GenBank (MN620068.1)	China	Jiangsu	Nanjing	32.073142	118.608523	1 (A5)	–
Asia	UFFE (31,864)	China	Jiangxi	Xiangshan	27.554360	116.039123	1 (A6)	1 (group 4)
Asia	UFFE (33,225, 33,226)	China	Shanghai	Shanghai Botanical Garden	31.148935	121.441839	5 (A1)	10 (group 1)
Asia	GenBank (MT178811.1)	India	Karnataka	Cottabetta	13.729165	75.574106	1 (B1)	–
Asia	GenBank (KY172634.1, KY172635.1)	India	Karnataka	Mudigere	13.08	75.63	2 (B2)	–
Asia	GenBank (MN620069.1)	Japan	Okinawa	–	26.344871	127.801188	1 (A3)	–
sia	GenBank (KU727031.1)	Vietnam	Phú Yên	Tuy Hoa	13.112984	109.277958	1 (B2)	–
Europe	INRAE team	France	Corsica	Cotti	41.772022	8.773401	2 (A1)	–
Europe	INRAE team	France	PACA	Cap d'Ail	43.728410	7.402091	1 (A1)	1 (group 1)
Europe	INRAE team	France	PACA	Cap Ferrat	43.676092	7.329719	4 (A1)	5 (group 1)
Europe	INRAE team	France	PACA	Château-Léoube	43.122588	6.275151	6 (A1)	7 (group 1)
Europe	INRAE team	France	PACA	Garoupe	43.563996	7.124504	2 (A1)	5 (group 1)
Europe	INRAE team	France	PACA	Le Lavandou	43.154	6.413	2 (A1)	–
Europe	INRAE team	France	PACA	Nice	43.695849	7.267888	4 (A1)	4 (group 1)
Europe	INRAE team	France	PACA	Sainte-Marguerite	43.519819	7.048594	2 (A1)	2 (group 1)
Europe	INRAE team	France	PACA	Saint-Tropez	43.261871	6.645200	1 (A1)	1 (group 1)
Europe	INRAE team	France	PACA	Villa Thuret	43.563996	7.124504	6 (A1)	5 (group 1)
Europe	Massimo Faccoli	Greece	Peloponnese	–	37.349380	22.352093	8 (A1)	8 (group 1)
Europe	INRAE team	Italy	Latina	Circeo Park	41.297216	13.046848	4 (A1)	11 (group 1)
Europe	Giovanna Tropea	Italy	Sicily	Donnafugata	36.881903	14.563506	2 (A1)	5 (group 1)
Europe	Giovanna Tropea	Italy	Sicily	Donnalucata	36.766307	14.636295	2 (A1)	5 (group 1)
Europe	Giovanna Tropea	Italy	Sicily	Marina di Ragusa	36.785731	14.548371	5 (A1)	5 (group 1)
Europe	Diego Gallego	Spain	Tarragonès	Vila-seca	41.11125	1.13381	4 (A1)	-
North America	UFFE (11,379)	USA	Florida	Austin Cary Forest	29.749605	−82.212870	5 (A2)	5 (group 2)
North America	UFFE (20,588, 20,595)	USA	Florida	Highlands Hammock Park	27.471267	−81.531776	6 (A2)	5 (group 2)
Pacific Island	GenBank (KX055191.1)	France	French Polynesia	Tefarerii	−16.79	−150.962	1 (A2)	-
Pacific Island	Jared Bernard	USA	Hawaii	Kaua'i Coffee Cie	21.31564	−157.80398	5 (A2)	3 (group 2)

Table 1 (continued)

Range	Sender (GenBank accession number/uffeID)	Country	State/Province/Region	Locality	Latitude	Longitude	No. in COI analysis (haplotype)	No. in RAD analysis (group)
Pacific Island	GenBank (KX818316.1 to KX818319.1)	USA	Hawaii	Kona Research Farm	22.198215	−159.334457	4 (A2)	-
Pacific Island	Jared Bernard	USA	Hawaii	Mānoa Valley	21.483681	−158.022090	5 (A2)	6 (group 2)
Pacific Island	Jared Bernard	USA	Hawaii	Moloa'a Coffee	19.6359	−155.95	2 (A2)	–
Pacific Island	UFFE (27,780)	USA	Hawaii	O'ahu	21.899523	−159.560966	1 (A2)	3 (group 2)
South America	GenBank (GU808706.1)	Brasil	Espirito Santo	–	–19.179798	−40.318079	1 (A2)	–
South America	UFFE (17,769)	France	French Guiana	Amazon Lodge	4.559321	−52.207490	4 (A2)	5 (group 2)
South America	UFFE (31,702)	France	French Guiana	Carrefour de Gallion	4.824021	−52.486669	1 (A2)	1 (group 2)

the NucleoSpin Gel and PCR Cleanup kit (Machery-Nagel) and sequenced in both directions using the ABI Prism BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI Prism 3500 Genetic Analyzer (Thermo Fisher Scientific). We used CodonCode (CodonCode Corporation) to check electropherograms, create contigs and trim all sequences to 566 bp. DNA sequences were aligned using ClustalW in MEGA X (Kumar et al. 2018). We completed the alignment with all the barcode COI sequences publicly available from Genbank and for which location information was available. Thus, 14 sequences from 10 locations in 8 countries were added, including sequences from Mitchell and Maddox (2010) (KX818316, KX818319), Cognato et al. (2020) (MN620067, MN620068, MN620069), Dole et al. (2010) (GU808707), Stouthamer et al. (2017) (KU727031.1) and Kiran et al. (2019) (KY172634.1, KY172635.1). The final alignment hence included 110 individuals (Table 1).

Mitochondrial data statistical analysis

We calculated Kimura 2 Parameters (K2P) genetic distances between haplotypes using MEGA X (Kumar et al. 2018). Haplotype and nucleotide diversities were calculated using the pegas package (Paradis 2010) in the R Software (R Core Team 2018). We reconstructed a phylogeny between haplotypes using Maximum Likelihood and Bayesian inference, with *X. germanus* and *X. crassiusculus* as outgroups (accession numbers NC036280.1 and MT230099.1, respectively). A Maximum Likelihood phylogeny was performed with MEGA X (Kumar et al. 2018) with 1000 bootstraps using K2P distances. A Bayesian inference of the haplotype phylogeny was performed with MrBayes (Ronquist et al. 2012) with a GTR + I + Γ evolutionary model, and four chains run four times during 2,000,000 generations with a diagnostic every 100 generations. A median-joining network was realised with PopArt (Bandelt et al. 1999). Haplotype maps were performed using the R packages maps (Becker et al. 2018), ggplot2 (Wickham 2016) and scatterpie (Guangchuang 2020).

RAD sequencing

DNA quantity and quality were assessed using the Qubit dsDNA HS Assay Kit with a Qubit fluorometer. As the DNA amount obtained from each individual was too small for the construction of RAD libraries, we followed the protocol used by Cruaud et al. (2018) to perform a whole genome amplification of each individual DNA sample with the Genomiphi kit V3 following the manufacturer's procedure. Individual RAD libraries were then constructed following Baird et al. (2008) and Etter et al. (2011) with a few modifications listed hereafter. DNA was digested using 250 ng of DNA in 22 µL per sample and 0.5 µL of the PstI-HF enzyme for a total volume of 25 µL. The digested fragments from each specimen were tagged with a unique 5- or 6- bp barcode and a P1 adapter using 1.5 µL of P1 adapter (100 nM) and 0.5 µL of T4 Ligase (2.000.000 U/ml) for a total volume of 30.5 µL. Specimens were then pooled 19 by 19 to create seven libraries. Libraries were sonicated on a Covaris S220 (duty cycle 10%, intensity 5, 200 cycles/burst, duration 75 s) to obtain 300–600 bp fragments. Each library was then tagged with a 5- or 6- nucleotide barcode and a P2 adapter using 1 µL of P2 adapter (10 nM) and 0.5 µL of Quick Ligase (2,000,000 U/ml). The sizing and purification steps were realised using AMPure XP beads (Agencourt). We performed 5 PCR enrichment with 15 cycles (30 ng DNA input, NEB Phusion High-Fidelity PCR Master Mix) for each library to increase fragment diversity. After quality control using the Agilent 2100 Bioanalyzer, the libraries were pooled altogether at an equimolar ratio and sent to MGX-Montpellier GenomiX for sequencing. The library was verified on a Fragment Analyser

(Agilent, HS NGS fragment Kit), quantified by qPCR (Kapa Library quantification kit) and sequenced on a SP lane in paired-end 2 × 150 nt mode on a Novaseq6000 (Illumina) according to the manufacturer's instructions.

RAD sequence data processing

We used the RADIS pipeline (Cruaud et al. 2016) to (i) demultiplex individuals using process_radtags (Catchen et al. 2013), (ii) homogenise read length and remove a few low-quality bases at the 3'-ends by trimming reads to 139 bp and (iii) remove PCR duplicates using clone_filter (Catchen et al. 2013). The following steps were performed using STACKS (Catchen et al. 2013; Rochette et al. 2019) on the Genotoul Bioinformatics Platform (INRAE, Toulouse, France). We tested two values of the M parameter from *ustacks* (i.e. the maximum distance allowed between stacks) M = 6 and M = 8. We also tested two values of the n parameter from *cstacks* (i.e. the number of mismatches allowed between sample loci when building the catalogue) n = 4 and n = 6. In order to remove potential fungal contaminations, we aligned the obtained loci on the *Ambrosiella xylebori* (*X. compactus*' symbiotic fungus) reference genome (Vanderpool et al. 2018) (accession number: ASM277803v1) using the BWA-MEM algorithm (Li and Durbin 2009) to create a loci blacklist that we later used to filter the fungus' reads in STACKS' *populations* module. In STACKS' *populations* module, we used three filtering values for parameter r (the minimum percentage of individuals required to process a locus, here with one population) r = 0, 0.5 and 0.7. We compared the number of SNPs obtained for each of the M, n and r Stacks parameters combinations. We also performed Principal Component Analyses (PCAs) and clustering with SNPrelate (Zheng et al. 2012) for the four M and n parameter combinations for r = 0.7. We excluded loci with a mean read depth lower than 8 using VCFtools (Danecek et al. 2011). We did not apply filtering based on minor allele frequency (we kept all SNP with at least one allelic variation) in order to avoid biasing subsequent statistical analyses (Linck and Battey 2019).

Lastly, we also wanted to determine the genetic structure of the symbiotic fungus. We thus ran the STACKS' *population* module using the loci mapping on the *Ambrosiella xylebori* reference genome as a whitelist, using r = 0.3 and excluding loci with a mean depth lower than 4.

RAD SNP statistical analysis

We estimated the specimens' relative ancestry using Admixture (Alexander et al. 2009), with a putative number of populations, K, ranging from 1 to 12 with a 100-fold cross-validation to assess the best K. We then used the pong 1.4.9 software (Behr et al. 2016) to estimate the major mode (using a greedy approach with 100 runs and a similarity threshold value of 0.90) and plotted the results using the package pophelper (Francis 2017) in the R Software (R Core Team 2018). A Maximum Likelihood tree was generated using RAxML 8.2.21 (Stamatakis 2014). We used the GTR CAT approximation and allowed the program to automatically halt bootstrapping using the bootstrap converge criterion (Pattengale et al. 2010) through the autoMRE option. The tree was visualised using FigTree V.1.4.4 (https://github.com/rambaut/figtree/releases). Besides, a hierarchical clustering tree was built using SNPRelate (Zheng et al. 2012) on an individual dissimilarity matrix (Zheng 2013). We also calculated the pairwise Fst (Weir and Cockerham 1984; Wright 1951) and Nei distances (Nei 1972) between the different groups obtained with the methods mentioned above using the StAMPP package (Pembleton et al. 2013). We also estimated the relative ancestry of the symbiotic fungi *Ambrosiella xylebori* and built a hierarchical clustering tree using the same procedure as for *X. compactus*.

Results

Mitochondrial diversity and differentiation

We obtained nine haplotypes worldwide (Fig. 1a–c, Table 1), with 70 variable sites out of 566 bp. Eight haplotypes were found in *X. compactus'* native area, including 5 in China (A1, A4, A5, A6 and C), 1 in Japan (A3), 1 common to India and Vietnam (B2) and 1 only in India (B1). Only three haplotypes were found in *X. compactus*' invaded range. The haplotype B2 was found in the two African countries studied, Uganda and Ghana. Only the haplotype A1 was observed in the invaded sites of southern Europe, this haplotype being also present in Shanghai but not in the site of the close Jiangsu province (Nanjing) nor the other sampled provinces. The haplotype A2 was found only in the invaded range and was present in every locality sampled in the Americas and the Pacific Islands. The K2P genetic distances suggested that the haplotypes could be divided into three groups with distances within groups lower than 0.045 (mean 0.018) and between groups higher than 0.056 (mean 0.0648) (Table 2). The first group (lineage A) comprised six haplotypes (A1 to A6) and was present in China, Japan, Europe, the Americas, Hawaii and French Polynesia. The haplotypes A1 and A2, the only haplotypes found in most of the invaded range, differed only by one substitution (Fig. 1c). The second group (lineage B) comprised two haplotypes (B1 and B2) and was found in Vietnam, India, Uganda and Ghana. The third group (lineage C) consisted of only one haplotype found in Hong Kong, whose sequence was published in a recent study by Cognato et al. (2020).

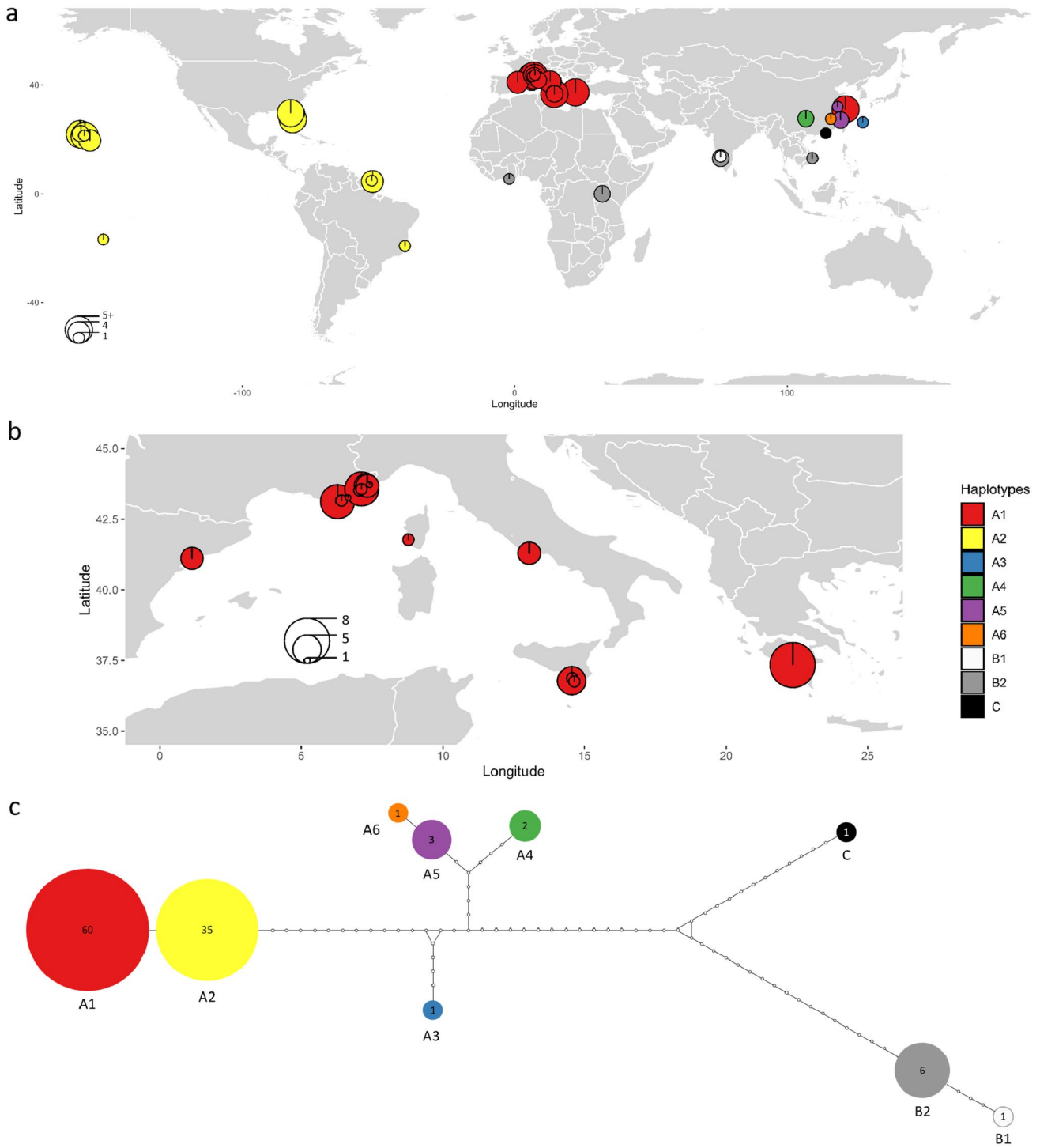

Fig. 1 *Xylosandrus compactus* haplotype maps (**a** and **b**) and median-joining network (**c**) based on COI sequences. The diagrams represent the sampling size and the proportion of each haplotype in each locality sampled **a** worldwide, and **b** focusing on Europe

We found only one haplotype in each locality; thus, haplotype and nucleotide diversities and their respective standard deviations at the locality level all equalled zero. The Maximum Likelihood tree reached high support values for every node and set the groups A and C in the same clade. The Bayesian inference tree had a lower resolution but placed groups B and C in the same clade with a significant posterior probability (Supplementary Figure S1).

Table 2 Genetic distances between COI haplotypes based on the Kimura 2-parameter model

	A1	A2	A3	A4	A5	A6	B1	B2
A1								
A2	0.002							
A3	0.033	0.031						
A4	0.044	0.042	0.027					
A5	0.042	0.040	0.025	0.013				
A6	0.040	0.038	0.023	0.011	0.002			
B1	0.086	0.084	0.068	0.070	0.071	0.070		
B2	0.082	0.080	0.064	0.066	0.067	0.066	0.007	
C	0.073	0.071	0.065	0.063	0.065	0.063	0.063	0.056

Genetic structure at nuclear SNPs obtained from RAD sequencing

We obtained a total of 563,419,874 reads, with an average of 2,324,174 (445,028 SD) reads per specimen after demultiplexing, and of 1,382,550 (252,282 SD) after removing low-quality reads and PCR duplicates. Depending on the combinations of the parameters M and n, 1.65 to 1.69% of the sequences mapped on the *Ambrosiella xylebori* genome and were blacklisted. The combination used of the parameters M and n had limited effects on our results. Indeed, each parameter combination of M, n and r yielded very similar numbers of loci, variant sites, and observed homozygosity (Supplementary Table 1). This was expected given the relatively low heterozygosity of the genome of species with regular inbreeding (Kirkendall et al. 2015). The Principal Component Analyses and the clustering analyses also gave similar results for the different parameter combinations (Supplementary Figure S2, Supplementary Figure S3). We decided to focus exclusively on the results obtained with $M=6$ and $n=4$, excluding loci with a mean depth lower than 8 or shared by less than 70% of the specimens ($r=0.7$). This M and n parameter set corresponds to the parameters used by Storer et al. (2017), adjusted for the read length. With these selected parameters values, we kept 27,583 SNPs.

The average homozygosity was 0.99 (0.002 SD), and the average inbreeding coefficient was 0.899 (0.02 SD). Using Admixture to explore the worldwide genetic structure of *X. compactus*, the cross-validation values reached a plateau for $K=4$ (Supplementary Figure S4). Increasing K above 4 increased the model's complexity at the cost of the geographical signal (Supplementary Figure S5); we thus selected $K=4$ as the most parsimonious number of genetic groups. With a similarity threshold of 0.90, the 100 Admixture runs yielded 24 different modes, the major mode representing 77 of them, with a pairwise similarity of 0.999. In all 233 runs of the major mode, all specimens were assigned to one of the four groups with a score higher than 0.95, and most of them scored more than 0.999 (Fig. 2). All specimens from Shanghai and Europe were assigned to group 1. The specimens from the Americas and Hawaii clustered together in group 2. The two remaining groups corresponded to the individuals from Uganda, Africa (group 3) and the other Chinese localities (group 4). Groups 1 and 2 were the closest, with a genetic distance of 0.03 (Table 3), while the genetic distances between group 4 and groups 1 and 2 were seven times larger. Group 3 was the most distant, with a genetic distance over 1 when compared with any of the three other groups. Despite the small number of specimens in group 4, the third-best mode split it into two groups. The RAxML analysis stopped after 400 bootstraps with a best tree scoring a GAMMA-score of -91,723.68 and depicting a genetic structure consistent with the Admixture results. Indeed, the Maximum Likelihood tree clearly divided the same four groups, respectively, Uganda, Europe and Shanghai, the Americas and Hawaii and the remaining localities in China. This analysis also showed a higher genetic differentiation between individuals from the different locations in Asia compared to between individuals within each of the three other clusters. The hierarchical clustering tree on the individual dissimilarity matrix yielded a similar genetic structure between samples (Supplementary Fig. 3).

Using the blacklisted reads as a whitelist to focus on the symbiotic fungus, we obtained only 95 SNPs after filtering with $r=0.3$ and excluding loci with a mean depth lower than 4. As for *X. compactus*, the cross-validation values reached a plateau for $K=4$ (Supplementary Material S6). With a similarity threshold of 0.90, the 300 Admixture runs yielded 83 different modes, the major mode representing 191 of them, with a pairwise similarity of 0.949. The analyses distinguished the same four groups as for the insect symbiont. However, in some runs, a few samples from group 1 were assigned to group 2 and reciprocally, most probably resulting from restricted power due to the limited number of loci (Supplementary Table S6).

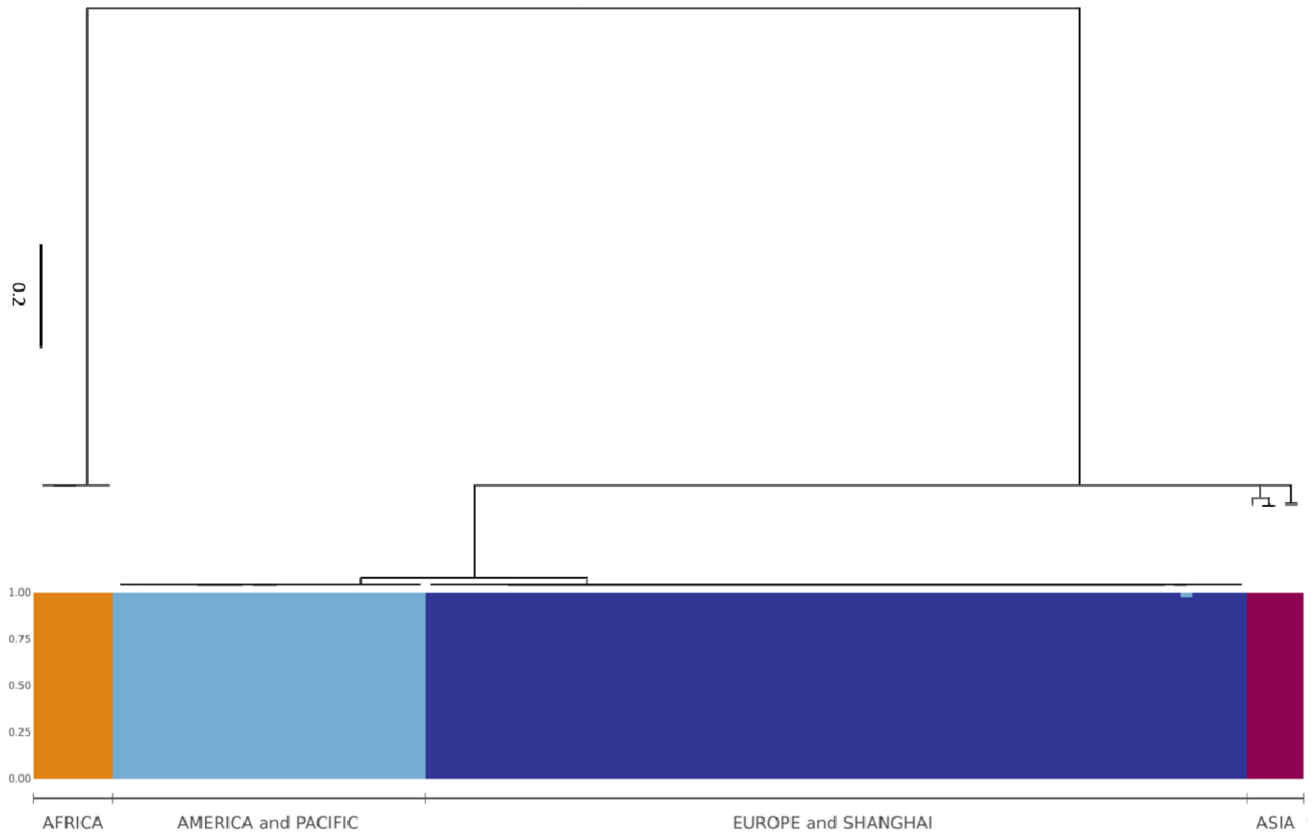

Fig. 2 Maximum likelihood tree performed with RAxML 8.2.1 and admixture plot for K = 4 calculated on RAD sequencing data. Specimens are divided according to their geographical origin

Table 3 Pairwise Fst (lower part) and Nei distances (upper part) calculated on RAD sequencing data between the four groups identified by Admixture

	Americas and Pacific	Africa	Europe and Shanghai	Asia
Americas and Pacific	0	1.315	0.028	0.195
Africa	0.994	0	1.315	1.109
Europe and Shanghai	0.797	0.994	0	0.196
Asia	0.938	0.975	0.954	0

Discussion

Invasion history of *X. compactus* and plausible scenarios

In spite of a limited sampling obtained in the native range, our results suggest the existence of genetic diversity and differentiation among *X. compactus* populations in Asia, with at least three mitochondrial lineages. The geographical distributions of these genetic groups within the native range will need to be characterised, as well as the putative existence of unsampled additional lineages. We showed that two of these genetic groups were the sources of all the invasive populations. Lineage A independently colonised the Americas and the Pacific Islands in the 1960s and Europe in the 2010s, and lineage B colonised Madagascar and Africa in the early twentieth century. The regions invaded by mitochondrial lineage A (Europe, the Americas and the Pacific islands) were characterised by very low genetic diversity despite the use of mitochondrial and pangenomic markers and extensive sampling coverage of their large geographical extent. Our results showed only one mitochondrial haplotype and almost no genomic diversity within each invasive group. This contrasts with other invasive ambrosia beetles, such as *X. crassiusculus*, whose populations in the invaded range were genetically diverse (Storer et al. 2017). However, it is similar to *X. germanus*, in which a single COI lineage was responsible for the European and the American invasions, although it showed higher haplotypic diversity in both continents than *X. compactus* (Dzurenko et al. 2020). According to the genetic invasion paradox (Sax and Brown 2000), we should expect invasive populations that experienced founder effects or bottlenecks to suffer from reduced fitness and evolutionary potential. *X. compactus*'

populations, however, might not fulfil the conditions to be called paradoxical (Estoup et al. 2016) as they presumably do not experience inbreeding depression thanks to the purge of the genetic load through repeated inbreeding (Schrieber and Lachmuth 2017). Indeed, a previous experimental study using *X. germanus* showed signs of outcrossing depression but not of inbreeding depression in this species (Peer and Taborsky 2005). Andersen et al. (2012) showed that *X. morigerus* follows the general-purpose-genotype model (Baker 1965), with generalist rather than specialised lineages with different ecological niches. *X. compactus* invaded Europe, the Americas and the Pacific Islands with almost no genetic diversity, suggesting that it also follows the general-purpose-genotype model and comprises generalist lineages occurring in various environmental conditions.

The absence of mitochondrial and genomic diversity both in Europe and in America and the Pacific is striking and suggests a single introduction in each continent followed by stepping-stone extension and within-continent human-aided dispersion, possibly through national and international live plant trade or timber and wood packaging material transportation. Indeed, further expansions involved long-distance dispersal that the insect's natural dispersal capacities cannot explain. Still, the absence of mitochondrial and genomic diversity could also result from repeated introductions from a single primary source in Europe and the American-Pacific region. As closely related but distinct mitochondrial haplotypes and RAD genetic groups occur in each region, we suppose that both continents were colonised independently from a very similar source. However, the very low genetic diversity found prevented us from inferring *X. compactus*' invasion history after its first entry, and we can only propose hypotheses based on historical data and dates of first detections. Concerning Europe, *X. compactus* was first detected in Italy in 2011 (Garonna et al. 2012), three years before France (Roques et al. 2019), which suggests that Italy might be the origin of the first step of the European invasion. However, we cannot infer the exact movements of the pest that caused the subsequent invasions in Greece, Mallorca, continental Spain and more recently Corsica. The same is true for the American-Pacific invasion, where a single colonisation event from the native range probably occurred. *X. compactus* was first detected in North America in 1941 (Ngoan et al. 1976), in Hawaii in 1964 (Hara and Beardsley Jr 1979) and South America in 1979 (Wood 1980). Thus, the populations from North America could have acted as a source for invasions in Pacific Islands or South America. *X. compactus* can disperse more than 8 km between two flying seasons (Gugliuzzo et al. 2019), which would be enough to disperse actively between close sites (e.g. between sampling sites in Hawaii). Between remote places, however, its spread was probably human-mediated through international trade.

The analyses revealed a relatively low genetic differentiation at nuclear loci between populations in Europe versus in America and the Pacific, in addition to a single mutational step difference between their mitochondrial haplotypes. This suggests a recent divergence and potentially similar or geographically close origins but no recent gene flow between both. We did not find native specimens that would group with the specimens from the Americas and Pacific Islands, probably due to poor coverage of the *X. compactus*' native range. On the contrary, the analyses consistently grouped the specimens from Europe with those sampled in Shanghai. Shanghai is one of the most economically important cities in China and the busiest port globally, from which quantities of goods, including ornamental plants, are exported worldwide (UNCTAD 2020). We thus hypothesise that Shanghai could be the donor area of the European and the American-Pacific invasions, presumably through international plant trade, but the origin may be larger since the samplings were limited in Eastern China. *X. compactus* is one of the most frequent pests in Shanghai's urban forests (Liu et al. 2021; Gao et al. 2017), and it is unknown whether it is native to Shanghai or not. Therefore, it cannot be excluded that the specimens we analysed originated from other parts of China through the trade of ornamentals within the country. Indeed, Shanghai imported various tree species from other Chinese regions to increase its plant diversity (Wang et al. 2020). Thus, Shanghai could have simply acted as a bridgehead by exporting plants infested by *X. compactus* from Shanghai, or already infested plants produced elsewhere as it has been reported by Bras et al. (2019) for the invasive box tree moth, C*ydalima perspectalis*. Another hypothesis would be that Europe was invaded by specimens from the American-Pacific regions. However, this is less parsimonious as we did not find the haplotype A2 in Europe, nor the haplotype A1 in the American-Pacific. Plus, the haplotype A2 is the only one sequenced in a large region, suggesting that it was stable over the last decades during the invasion of the Americas and the Pacific Islands. Thus, it is unlikely that it mutated before reaching Europe and Shanghai, or that the only mutation between A1 and A2 appeared both in the American-Pacific and Shanghai.

Ghana and Uganda were invaded by the same mitochondrial haplotype belonging to the lineage B, presumably more than a century ago (Egonyu et al. 2015). The haplotype B2, which occurs in Africa, was observed within the native range in a locality in Vietnam and a locality in India, where Kiran et al. (2019) sequenced around 200 specimens and found the haplotype B2 exclusively. These two localities are more than 3500 km apart, and a more thorough sampling throughout Asia might have revealed the haplotype B2 in other localities, potentially along the Bay of Bengal. Unfortunately, Vietnam, India, and Ghana were not included in the

RAD sequencing experiment (Table 1), which could have helped propose hypotheses about Africa's invasion history. Although not much is known about the first steps of *X. compactus* invasion in Africa, we hypothesise that *X. compactus* might have been introduced to Africa from Southeastern Asia, maybe from India or Vietnam. Extensive sampling and genetic characterisation of *X. compactus* are needed to assess the number of lineages present in Madagascar and Africa and test whether genetic diversity is also almost non-existent in this continent.

Despite a very limited sampling of the native range, we found that populations from the native range carried a higher genetic diversity than in the numerous populations sampled in the invasive range. We can hypothesise that, similarly to *X. crassiusculus* and *X. germanus*, genetic diversity in native populations of *X. compactus* is relatively high, and further genetic structure may be identified with a more extensive sampling there. A comprehensive study of *X. compactus* genetic structure in its native range is needed to better describe its genetic diversity, to map the distribution of the different mitochondrial lineages and increase our understanding of *X. compactus*' evolutionary history and colonisation pathways. This would also allow to formally test whether the genetic diversity is almost non-existent at the local scale. Specimens from Shanghai excluded, Admixture assigned all Asian specimens to the group 4, despite larger differences between them than between specimens from the groups 1 and 2 (Fig. 2). This is most likely due to the difference of sample size between the larger groups 1 and 2, and group 4 (Table 1), and a study including more specimens from *X. compactus*' native range would presumably reach a higher number of clusters and notably split the group 3 in several groups, again arguing for a more extensive sampling of populations in the native range.

Our analysis revealed low individual heterozygosity and high Fst between genetic groups. This must be interpreted with caution, as restricted dispersal and hence low gene flow between populations, sib-mating and haplodiploidy contribute to very low heterozygosity and to a predominant effect of drift on differentiation between lineages. Hence, these metrics are expected to show particularly extreme values compared to organisms having higher dispersal and reproductive strategies implicating more random mating between local individuals and to diploid organisms. Nevertheless, such low heterozygosity and high differentiation are observed among a wide diversity of organisms, including insects (e.g. Eyer et al. 2018; Andreev et al. 1998). In the context of assessing invasion routes and identifying source locations of the invading lineages, such high Fst between lineages have one major advantage and one major inconvenient. The advantage is that given the depleted diversity and genetic stability of sib-mating populations, the several invading lineages can be deciphered efficiently and traced along their way. The disadvantage is that, as the potential source populations are highly differentiated, one has to genotype virtually almost all the source populations to be able to assign invading lineages to their source. This task would be much easier if populations were less differentiated and with a smoother pattern of isolation by distance (e.g. Wasser et al. 2004). Less variable DNA markers (i.e. mitochondrial or nuclear ultra-conserved elements) may be more suitable to attain such objective in the context of highly structured populations.

The analyses performed on the symbiotic fungus lacked power, probably because of the relatively small number of loci available (95 compared to 27,583 for *X. compactus*). Yet, they showed results consistent with those for *X. compactus*, with four different groups emerging from Admixture and the clustering tree, which suggests a parallel genetic structure, gene flow, and invasion history of the pest insect and its symbiotic fungus. As the genome of *Ambrosiella xylebori* is available, it would be interesting in future studies to extract DNA of both the insect and the symbiotic fungus separately, to obtain more markers from the fungus and confirm the parallel histories of both partners.

Management implications

The objective of invasive pest management is to lower the damage resulting from the invasion. This can be accomplished by preventing the invasion, eradicating an incipient invasion, or adapting the management of nurseries, orchards, and forests to the new pest.

In terms of prevention of *X. compactus* invasion, it is no longer possible for the Americas, Europe and many islands. Once established, the eradication of *X. compactus* is likely impossible. Indeed, given in the absence of the Allee effect in the population dynamics of inbreeding ambrosia beetles, eradication would require the elimination of all individuals. Invasion eradication has succeeded in the case of *Xylosandrus crassiusculus* in Oregon, but only while its distribution was restricted to one small area (LaBonte 2010).

X. compactus is not yet established in the entirety of the suitable area and could presumably colonise new countries such as Australia or New Zealand and pursue its expansion in its invaded range, notably in the Mediterranean (Urvois et al. 2021). The results we presented here showed that both mitochondrial and nuclear markers revealed the same invasion history without discrepancy and could be used to monitor and screen for the pest invasion in regions where it does not occur yet. Although RAD sequencing could be helpful to understand the finer genetic structure in *X. compactus*' native range, its development is demanding, and it is not necessary for detection and monitoring tools given the low diversity in the invaded ranges. We advise to use mitochondrial DNA sequencing to survey *X. compactus*

in the invasive range, as it is faster, less expensive and requires less equipment. As our results point to a single source for both colonisations of Europe and the Americas, we suggest a more thorough screening of imports originating from the region of Shanghai, mainly live plants or wood packaging, as it may be the source of the invasion of *X. compactus* in most of the world. Rizzo et al. (2021) developed a protocol allowing the identification of *Xylosandrus compactus*, *X. germanus* and *X. crassiusculus* from a segment of gallery or frass. This could be of great help to identify the species after the specimens have dispersed from the galleries. We also advise border protection agencies to deploy traps with attractive lures (a combination of UHR Ethanol, α-pinene, α-copaene and quercivorol, Roques et al., in prep) in ports of entry and to routinely sequence the specimens' mitochondrial DNA to characterise their lineage and haplotype to identify their origin. In already invaded areas, this could help detect additional invasions from new sources, which is a valuable information as populations from different origins could display different behaviours or have different ecological preferences, ultimately affecting management success. Identifying the source of the invasion would also help target the best agent in the case of classical biological control.

In countries where the species is established, there are only limited options for direct control of the pests. Fortunately, as with other *Xylosandrus*, *X. compactus* also attacks stressed trees more than healthy trees (Ranger et al. 2015). Therefore, improving tree health and ecosystem health is an effective management approach, particularly in terms of irrigation (Gugliuzzo et al. 2021).

Author's contribution statement

C.K., A.R. and M.A.A.R. designed the study. L.S., C.C. and T.U. completed the molecular biology work. T.U. and C.P. performed the bioinformatics, the statistical analyses and made the figures. Y. L., A.J.J. and J. H. organised the field work. T.U. wrote the original draft of the manuscript. All authors reviewed, edited and approved the final version of the manuscript.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10340-021-01443-7.

Acknowledgements We would like to thank Richard Bellanger, Jared Bernard, Jean-Baptiste Daubrée, Jacques Deleuze, Massimo Faccoli, Steve Frank, Lei Gao (Shanghai Academy of Landscape Architecture Science and Planning), Benjamin Gey, Federica Giarruzzo, Fabrice Pinard, and Giovanna Tropea for sending specimens. We also would like to thank Anne Loiseau for the laboratory work. We are grateful to MGX-Montpellier GenomiX for sequencing the RAD libraries and to the Genotoul bioinformatics platform Toulouse Occitanie (Bioinfo Genotoul, https://doi.org/10.15454/1.5572369328961167E12) for providing computing and storage resources. We are also grateful to the Sino-French Joint Laboratory for Invasive Forest Pests in Eurasia (IFOPE) for its support.

Funding This work was supported by the LIFE project SAMFIX (SAving Mediterranean Forests from Invasions of *Xylosandrus* beetles and associated pathogenic fungi, LIFE17 NAT/IT/000609, https://www.lifesamfix.eu/) which received funding from the European Union's LIFE Nature and Biodiversity programme. JH and AJJ were supported by the US Forest Service, the USDA APHIS, and the National Science Foundation.

Availability of data and material Individual RAD sequence files are available in a fq.gz format at the Sequence Read Archive (SRA) (Study Accession no. PRJNA771401). The VCF files Xylosandruscompactus.vcf and Ambrosiellaxylebori.vcf used for population genomic analyses, as well as the popmap used in STACK' population module and specimens' metadata (e.g., GPS coordinates) are available on Portail Data INRAE (https://doi.org/10.15454/ETBWEP). The Genbank accession numbers for the mitochondrial haplotypes reported in this paper are OK489329:OK489334.

Declarations

Conflict of interest The authors declare no conflict of interests. Specimens sampled did not involve endangered nor protected species.

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