J Pest Sci (2019) 92:173–187
DOI 10.1007/s10340-017-0917-1

ORIGINAL PAPER

Complex invasion history of the Asian long-horned beetle: fifteen years after first detection in Europe

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Received: 29 May 2017 / Revised: 5 September 2017 / Accepted: 13 September 2017 / Published online: 19 September 2017

Abstract The Asian long-horned beetle (ALB), a Cerambycidae, is an urban tree pest native to East Asia accidentally introduced to other continents via solid wood packing material. It was first detected in Europe in 2001, and since then infestations have been found in ten European countries. Using a 485-bp-long fragment of the mitochondrial barcode gene (COI), we studied the genetic diversity and structure of ALB populations in both native and invaded ranges, with a specific focus on Europe. Three main haplotypes were found across the native and invaded distribution of ALB. The native area in Asia was the most diverse with 23 haplotypes, but a low genetic structure was observed. Our results revealed up to nine distinct haplotypes that was diverged by no more than six mutational steps in European populations collected from 2001 to 2016. Nevertheless, the genetic structure was characterized by one widespread dominant haplotype in Europe. The overall complex genetic structure observed in Europe suggested a convoluted invasion scenario. Indeed, invasion history may include several introduction events as well as secondary dispersal.

Keywords Biological invasion · *Anoplophora glabripennis* · ALB, genetic structure · Mitochondrial marker · Cytochrome oxidase I (COI)

Communicated by J. D. Sweeney.

Special issue on "Invasive insect pests of forests and urban trees: pathways, early detection and management".

Electronic supplementary material The online version of this article (doi:10.1007/s10340-017-0917-1) contains supplementary material, which is available to authorized users.

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Key messages

- The Asian long-horned beetle (ALB) *Anoplophora glabripennis* is a significant invasive pest of urban trees in Europe and North America.
- We investigated the genetic structure of this species in both invaded and native ranges, with a specific focus on the European outbreaks, using the mitochondrial COI gene.
- Our results showed that the patterns of introduction of ALB in Europe are complex, and potentially include multiple introduction events coupled with secondary spread within the invaded range.
- These data provide crucial information to optimize and target management measures, as well as prevent further introductions.

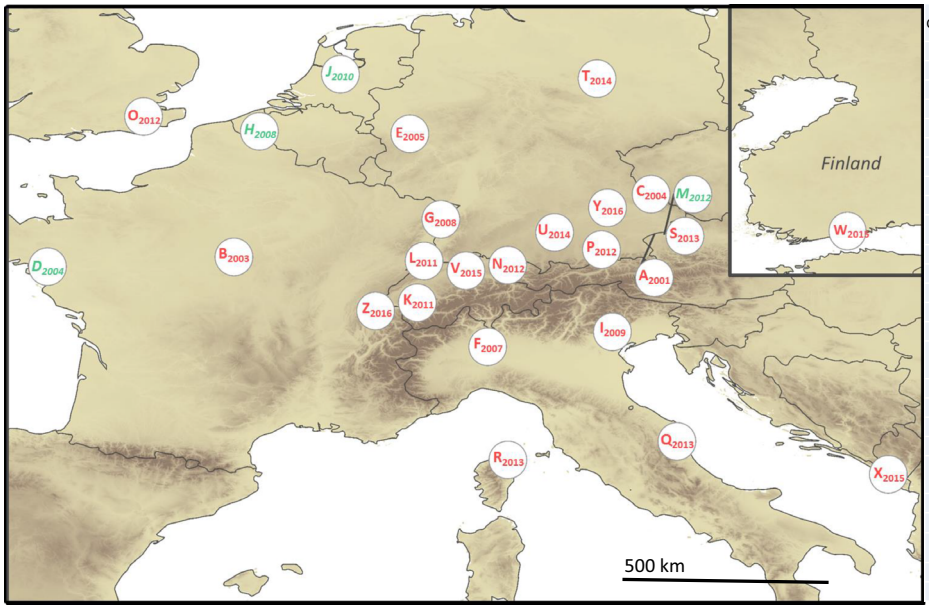
Introduction

Many studies show large increases in the introduction rates of invasive alien species (IAS) in the 19th and 20th centuries, and the worldwide acceleration of invasions was

shown to be concomitant with the increasing intercontinental and regional trade favoring entry and dissemination (e.g., Roques et al. 2016), changes in host plant distributions (e.g., Hurley et al. 2016), and with the climate change (Walther et al. 2009; Ramsfield et al. 2016). However, patterns differ between regions and considered taxa during recent decades (Hulme 2009; Aukema et al. 2010; Roques 2010; Hurley et al. 2016). In Europe, the annual mean number of newly recorded alien insect species has nearly doubled in a little less than half a century (Roques 2010). In addition, once established in Europe, most alien insects appear to spread much more rapidly than prior to the 1990s (Roques et al. 2016). Indeed, the recent changes in European political and economic policies combined with the liberalization of trade and travel are hypothesized to facilitate the dissemination of invasive alien species once they are introduced (Roques et al. 2016).

The genetic structure of an invasive population usually reflects its introduction history, which is mainly defined by the number of introductions events, the steps on the introduction pathways and the number of propagules introduced at each step (Dlugosh and Parker 2008; Garnas et al. 2016). Multiple introductions are, for example, a common scenario for IAS, and have been identified for several insect species (e.g., Lombaert et al. 2010; Ciosi et al. 2008). These multiple introduction events can strengthen invasive populations since they can help maintaining a diversity comparable to or even greater than that of the native zone (Dlugosch and Parker 2008; Roman and Darling 2007; Estoup et al. 2016; Roques et al. 2016). This is especially true if the additional introductions come from a genetically differentiated population (Dlugosh and Paker 2008; Rius and Darling 2014), as it increases the probability of introducing genetically different individuals. The potentially higher resulting diversity can counterbalance the deleterious effects of genetic drift or inbreeding depression and may contribute to the settlement and persistence of an invasive alien species (IAS) by increasing their ability to adapt to their new environment (Estoup et al. 2016). Another invasion scenario is the bridgehead effect in which a successfully introduced population acts as the source of the colonists that invade another area (Lombaert et al. 2010). This has already been demonstrated for several species, and it seems to be common for IAS (Yang et al. 2012; Lombaert et al. 2010; Hurley et al. 2016; Garnas et al. 2016). Deciphering population structure of native and invasive populations is moreover a crucial step for identifying an IAS, managing established populations and preventing further introductions (Estoup and Guillemaud 2010; Essl et al. 2015). These management practices need to be adapted for the invasive population of the targeted IAS, and the management of introduced populations requires a better understanding of the dynamics of the invasion itself, with an assessment of parameters such as the number of introduction events, the frequency of new introductions or the relationships between different populations both in the native and invaded ranges (Roderick and Navajas 2003).

The Asian long-horned beetle (ALB) *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), native to China and Korea (Lingafelter and Hoebeke 2002) is listed among the one hundred worst IAS in the world (Lowe et al. 2000). In its native range, ALB has primarily been a pest of tree plantations, windbreaks and urban trees (Pan 2005). In native hosts of Korea, ALB is found at fairly low densities in forested riparian areas where it is not a pest (Williams et al. 2004a). ALB infestations and range expansion in Asia were shown to be linked to widespread afforestation and reforestation efforts in China since the 1960s (Haack et al. 2010). The constant increase in international trade from Asia since the early 1980s (Normile 2004) has likely increased the probability of its spread, given that ALB larvae can be transported hidden within untreated solid wood packing material (Bartell and Nair 2003; Hu et al. 2009; Haack et al. 2010). As a result, established populations of ALB have been reported in North America since 1996 (Haack et al. 1996) and in Europe since 2001 (Hérard et al. 2006). However, the year of detection does not necessarily correspond to the year of introduction, as it may take several years after introduction before the first signs of the infestation are detected (Favaro et al. 2013). In North America, ALB was detected for the first time in 1996 in the USA (Haack et al. 1996) and in 2003 in Canada (EPPO Global Database), and is still under eradication (Haack et al. 2010). In Europe, since the first record in Austria in 2001, additional infestations have been detected in other countries including France (2003), Germany (2004), Italy (2007), Belgium (2008), the Netherlands (2010), Switzerland (2011), UK (2012), Finland (2015) and Montenegro (2015) (EPPO Global Database). Despite the eradication programs initiated in Europe (Haack et al. 2010), ALB populations are still reproducing and potentially spreading in some parts of Europe (Fig. 1). This wood-boring insect can damage and ultimately kill a large number of tree species. Its life history traits and ecology have been recently reviewed by Meng et al. (2015). Briefly, females lay their eggs under the bark of apparently healthy deciduous trees (Haack et al. 2010; Meng et al. 2015). Adults do not disperse far and tend to stay in the vicinity as long as host quality for the larvae is adequate (Sawyer 2009; Hu et al. 2009). In its native range, most ALB infestations are seen in urban environments and in monoculture stands (Smith et al. 2009). In Europe, no population has been detected in natural forests so far, the beetles being found only in urban environments (Hérard et al. 2006). The situation is similar in North America with



Code	Locality	Country	First detection	Eradication
A	Braunau	Austria	2001	2013
B	Gien	France	2003	/
C	Neukirchen	Germany	2004	2016
D	St Anne sur Brivet	France	2004	2008
E	Bornheim	Germany	2005	/
F	Corbetta	Italy	2007	/
G	Strasbourg	France	2008	/
H	Courtrai	Belgium	2008	2011
I	Cornuda	Italy	2009	/
J	Almere	Netherlands	2010	2016
K	Brünisried	Switzerland	2011	/
L	Weil-am-Rhein	Germany	2011	/
M	Geinberg	Austria	2012	2017
N	Winterthour	Switzerland	2012	2017
O	Paddock Wood	UK	2012	/
P	Feldkirchen bei München	Germany	2012	/
Q	Grottazzolina	Italy	2013	/
R	Furiani, Corsica	France	2013	/
S	Gallspach	Austria	2013	/
T	Magdeburg	Germany	2014	/
U	Ziemetshausen-Schönebach	Germany	2014	/
V	Berikon	Switzerland	2015	/
W	Vantaa	Finland	2015	/
X	Budva	Montenegro	2015	/
y	Kelheim	Germany	2016	/
Z	Divonne-les-Bains	France	2016	/

Fig. 1 Record of the distribution of ALB in Europe since its first detection in 2001. The active infestations to date are represented in red and bold; the eradicated infestations are in green and italic. Infestations reported at less than 20 km from each others are considered identical. Table on the right summarizes the codes reported on the map, the site associated and the dates of detection and eradication if relevant

the exception of a population detected in 2008 in a peri-urban forest in Massachusetts, USA (Dodds and Orwig 2011). This highlights the fact that ALB could be a threat for natural environments and that its management is of crucial importance.

To our knowledge, several studies of the genetic structure of ALB have been published focusing mainly on the native Asian and the invaded North American ranges (An et al. 2004; Carter et al. 2008, 2009, 2010). In the native range, Carter et al. (2009) reported a pattern of genetic admixture among Asian populations using mitochondrial DNA and microsatellites markers. They hypothesized that this pattern could be linked to the reforestation efforts to combat desertification in China, and they assumed that the natural dispersal abilities of ALB may have also played a role. More recently, Carter et al. (2010), using mitochondrial DNA and microsatellite markers showed that several introduction events may explain the observed genetic pattern in invaded areas, but their dataset mainly focused on North America and Europe was not fully investigated.

In this study, the genetic structure of European ALB populations was surveyed using the mtDNA cytochrome oxidase 1 (COI) gene. This gene has been widely used to study the phylogeography of endemic species (De-la-Mora et al. 2015; Drag et al. 2015), but also to trace invasions (Downie 2002; Yang et al. 2012; Auger-Rozenberg et al. 2012; Lippens et al. 2017). The aim of this study is to determine the genetic structure of the invasive populations and survey the genetic diversity observed among these populations, with specific attention to the European infestations. The genetic structure of ALB in Europe is compared with the patterns observed in the North American invaded range and the Asian native range. This work is the first comprehensive study analyzing almost 700 individuals from three continents, and will help to understand the invasion process as well as develop effective containment and management measures.

Materials and methods

Sampling and building dataset

Fresh ALB samples were collected in China in the summer of 2016 to broaden the Asian native range sampled beyond the specimens that were already available for DNA sequencing. Therefore, a total of 96 beetles from 17 sites were analyzed (Table 1). Additionally, the DNA from 55 specimens from 7 North American sites and 93 specimens from 20 European sites were sequenced (Fig. 2, Table 1). Most specimens were collected alive and preserved in 96% ethanol until DNA extraction; the others were stored frozen. Most insects were collected as adults, which made their identification easy. Immature stages were identified morphologically when possible, and the identification was checked by comparing their DNA sequence with the Genbank database, since *A. glabripennis* larvae can easily be confused with those of *A. chinensis*, a closely related

Table 1 Summary of sampling sites and common parameters of genetic diversity of the Asian long-horned beetle

	Country	State/province (closest town)	Date of collection	Longitude	Latitude	*n*	*N* variable sites	n ht	*h*	π	*r*
Total						696	25	23	0.715	0.00571	
Pop number (Fig. 2)	Asia					225	25	19	0.857	0.00788	
25	**China**	**Zhejiang (Cixi)**	**2015**	**121.26**	**30.16**	**11**	**6**	**3**	**0.473**	**0.00404**	**0.473**
26	**Korea**	**(Incheon)**	**2013–14**	**126.7**	**37.45**	**2**	**0**	**1**	**0**	**0**	**0**
27	**Korea**	**(Pocheon)**	**2015**	**127.2**	**37.89**	**6**	**9**	**2**	**0.333**	**0.00619**	**0.333**
28	**Korea**	**(Ulsan)**	**2015**	**129.31**	**35.53**	**4**	**0**	**1**	**0**	**0**	**0**
29	China	(Shanghai)		121.45	31.15	3	13	3	1	0.01787	**1**
30	China	Anhui (Wuhu)		118.35	31.33	3	3	2	0.667	0.00412	**0.667**
31	China	Anhui (Bengbu)		117.36	32.93	8	17	7	0.964	0.01289	**0.857**
32	China	Gansu (LiuHua)		103.26	35.95	2	1	2	1	0.00206	0
33	China	Gansu (Jingtai)		104.05	37.35	9	14	4	0.694	0.01237	**0.556**
34	China	Gansu (Lanzhou)		103.93	36.31	10	10	5	0.756	0.00724	**0.756**
35	China	Hebei (Langfang City)		116.66	39.5	11	2	2	0.509	0.0021	**0.509**
36	China	Hebei (HanDan/DA)		115.13	36.26	2	0	1	0	0	0
37	China	Hebei (HanDan/CH)		114.35	36.43	2	4	2	1	0.00825	1
38	China	Hebei (Chengguan)		116.48	37.6	3	1	2	0.667	0.00137	**0**
39	**China**	**Hebei (Shijiazhuang)**	**2015**	**114.95**	**38.16**	**5**	**4**	**3**	**0.524**	**0.00236**	**0.524**
						2					
40	China	Jilin (Lishe)		124.31	43.3	5	9	5	1	0.00948	1
41	China	Liaoning		123.43	41.83	2	0	1	0	0	0
42	China	Liaoning (Linghai)		121.36	41.15	2	0	1	0	0	0
43	China	Liaoning (XingCheng)		120.36	40.6	3	0	1	0	0	0
44	**China**	**Inner Mongolia (Hohhot)**		**111.63**	**40.8**	**10**	**0**	**1**	**0**	**0**	**0**
				111.63	40.8	9					
45	China	Inner Mongolia (Wuhai)		106.78	39.65	5	2	2	0.4	0.00165	0.4
46	China	Inner Mongolia (Hasa)		111.83	40.83	2	0	1	0	0	0
47	China	Inner Mongolia		105.95	38.81	3	4	2	0.667	0.0055	0.667
48	**China**	**Ningxia (Qingtongxia)**	**2015**	**105.95**	**37.86**	**5**	**4**	**3**	**0.733**	**0.00467**	**0.6**
						1					
49	China	Tianjin (Datian)		117.8	39.23	3	0	1	0	0	0
50	China	Tianjin (Chadian)		117.75	39.23	3	0	1	0	0	0
51	China	Tianjin (Jinghai)		116.9	38.93	2	0	1	0	0	0
52	China	Tianjin (Ninghe)		117.78	39.31	3	0	1	0	0	0
53	China	Shanxi (Taiyuan)		112.68	38.3	1	NA	NA	NA	NA	/
54	China	Shandong (Guanxian)		115.43	36.46	1	NA	NA	NA	NA	/
55	Korea	(Sokcho)		128.45	38.11	17	1	2	0.441	0.00091	0.441
80	China	Henan (Zhengzhou NE)		113.63	34.75	4	7	2	0.5	0.00722	0.5
81	China	Henan (Zhengzhou SM)		115.1	35.9	3	0	1	0	0	0
82	**Korea**	**(Kangwon)**		**128.7**	**38.21**	**15**	**0**	**1**	**0**	**0**	**0**
24	**China**	**Anhui (Hefei)**	**2016**	**117.2272**	**31.8205**	**4**	**9**	**2**	**0.667**	**0.0124**	**0.667**
83	**China**	**Shandong (Jinan)**	**2016**	**117.12**	**36.6512**	**5**	**3**	**2**	**0.4**	**0.00276**	**0.4**
84	**China**	**Shandong (Taian)**	**2016**	**117.0876**	**36.2002**	**5**	**5**	**3**	**0.8**	**0.00537**	**0.8**
85	**China**	**Ningxia (Yanchi)**	**2016**	**107.4073**	**37.7832**	**5**	**2**	**2**	**0.6**	**0.00248**	**0.6**
86	**China**	**Yunnan**	**2016**	**102.71**	**25.0458**	**5**	**0**	**1**	**0**	**0**	**0**
87	**China**	**Inner Mongolia (Tongliao)**	**2016**	**122.2434**	**43.6528**	**5**	**0**	**1**	**0**	**0**	**0**
88	**China**	**Jilin (Yanji)**	**2016**	**129.5089**	**42.8912**	**5**	**5**	**2**	**0.4**	**0.00414**	**0.4**

Table 1 continued

	Country	State/province (closest town)	Date of collection	Longitude	Latitude	*n*	*N* variable sites	n ht	*h*	π	*r*
89	**China**	**Hebei (Chengde)**	**2016**	**117.9627**	**40.9529**	**4**	**7**	**3**	**0.833**	**0.00893**	**0.833**
90	**China**	**Heilongjiang (Harbin)**	**2016**	**126.5349**	**45.8037**	**5**	**6**	**3**	**0.7**	**0.00702**	**0.7**
North America						322	8	7	0.715	0.00603	
1	**Canada**	**Ontario (Tallgrass)**		**−75.75**	**45.26**	**11**	**0**	**1**	**0**	**0**	**0**
2	**Canada**	**Ontario (Northview)**		**−79.54**	**43.79**	**4**	**1**	**2**	**0.5**	**0.00115**	**0.5**
3	**Canada**	**Ontario (Sheppard)**		**−79.87**	**44.16**	**3**	**0**	**1**	**0**	**0**	**0**
4	USA	New Jersey (Carteret)		−74.22	40.57	22	1	2	0.091	0.00019	**0**
5	**USA**	**New York (Bayside, NYC)**		**−73.76**	**40.75**	**14 6**	**2**	**2**	**0.505**	**0.00313**	**0.505**
6	USA	New York (Brooklyn, NYC)		−73.94	40.67	19	7	2	0.199	0.00287	**0.199**
7	USA	New York (Flushing, NYC)		−73.83	40.76	3	0	1	0	0	**0**
8	USA	New York (Forest Park, NYC)		−73.84	40.7	9	0	1	0	0	**0**
9	USA	New York (Kew Garden Hills, NYC)		−73.81	40.72	5	0	1	0	0	**0**
10	USA	New York (Long Island City, NYC)		−73.94	40.74	5	5	2	0.4	0.00412	**0.4**
11	USA	New York (Manhattan, NYC)		−73.97	40.78	18	0	1	0	0	**0**
12	USA	New York (Maspeth, NYC)		−73.9	40.72	14	2	2	0.143	0.00059	**0.143**
13	USA	New York (Mt Olivet Cemetry, NYC)		−73.89	40.72	9	0	1	0	0	**0**
14	USA	New York (Prall's Island)		−74.2	40.6	6	0	1	0	0	**0**
15	USA	New York (Staten Island)		−74.15	40.57	2	0	1	0	0	0
16	USA	New York (Sunnyside, NYC)		−73.91	40.74	5	0	1	0	0	0
17	USA	New York (Amityville)		−73.41	40.67	13	7	4	0.526	0.00455	0.526
18	USA	New York (Islip)		−73.21	40.72	2	0	1	0	0	0
19	USA	New York (Massapequa)		−73.47	40.68	16	2	2	0.458	0.00189	0.458
20	USA	New Jersey (Jersey City)		−74.07	40.72	14	0	1	0	0	0
21	USA	New Jersey (Linden)		−74.24	40.62	30	7	2	0.067	0.00096	0.067
22	**USA**	**Chicago (Ravenswood)**		**−87.68**	**41.96**	**3 12**	**0**	**1**	**0**	**0**	**0**
23	Canada	Ontario (Toronto)		−79.38	43.65	57	0	1	0	0	0
78	**USA**	**Massachusetts (Worcester)**		**−72.33**	**42.51**	**15**	**0**	**1**	**0**	**0**	**0**
79	**USA**	**Ohio (Bethel)**		**−84.13**	**39.61**	**5**	**0**	**1**	**0**	**0**	**0**
Europe						149	8	9	0.533	0.00199	
56	**Switzerland**	**Berikon**	**2015**	**8.37**	**47.35**	**17**	**1**	**2**	**0.118**	**0.00024**	**0**
58	**Switzerland**	**Bruenisried**	**2013–14**	**7.27**	**46.75**	**11**	**0**	**1**	**0**	**0**	**0**
59	**Italy**	**Cornuda**	**2014**	**12**	**45.83**	**5**	**1**	**2**	**0.6**	**0.00124**	**0.6**

Table 1 continued

	Country	State/province (closest town)	Date of collection	Longitude	Latitude	*n*	*N* variable sites	n ht	*h*	π	*r*
60	**France**	**Upper Corsica (Furiani AR)**	**2015**	**9.43**	**42.68**	**13**	**1**	**2**	**0.154**	**0.00032**	**0.154**
61	**France**	**Upper Corsica (Furiani MC)**	**2015**	**9.43**	**42.65**	**7**	**0**	**1**	**0**	**0**	**0**
62	**France**	**Upper Corsica (Furiani NVX)**	**2015**	**9.41**	**42.65**	**5**	**0**	**1**	**0**	**0**	**0**
92	**France**	**Upper Corsica (Furiani LST)**	**2016**	**9.42**	**42.66**	**5**	**0**	**1**	**0**	**0**	**0**
63	**France**	**Upper Corsica (Furiani STD)**	**2015**	**9.44**	**42.65**	**4**	**0**	**1**	**0**	**0**	**0**
64	**Germany**	**Ebersberg**		**11.96**	**48.07**	**1**	**0**	**1**	**0**	**0**	**/**
65	**Germany**	**Feldkirchen**		**11.73**	**48.14**	**5**	**0**	**1**	**0**	**0**	**0**
66	**Switzerland**	**Marly**	**2014**	**7.14**	**46.8**	**8**	**0**	**1**	**0**	**0**	**0**
67	**Austria**	**Gallspach**	**2014**	**13.8**	**48.21**	**4**	**0**	**1**	**0**	**0**	**0**
68	**France**	**Loiret (Gien)**	**2015**	**2.62**	**47.69**	**75**	**0**	**1**	**0**	**0**	**0**
69	**Germany**	**Magdeburg**		**11.62**	**52.12**	**61**	**1**	**2**	**0.286**	**0.00059**	**0.286**
70	**Germany**	**Neubiberg**		**11.67**	**48.07**	**82**	**4**	**3**	**0.378**	**0.00166**	**0.378**
71	**Italy**	**Rapagnano**	**2015**	**13.59**	**43.16**	**2**	**0**	**1**	**0**	**0**	**0**
72	**France**	**Loire Atlantique (St-Anne-sur-Brivet)**	**2004–05**	**−2**	**47.45**	**4**	**2**	**2**	**0.5**	**0.00207**	**0.5**
73	**France**	**Bas-Rhin (Strasbourg)**	**2010-11-14**	**7.75**	**48.57**	**3**	**0**	**1**	**0**	**0**	**0**
74	**Switzerland**	**Winterthur**	**2012**	**8.52**	**47.39**	**11**	**3**	**2**	**0.182**	**0.00126**	**0.182**
75	Austria	Braunau		13.04	48.25	4	1	2	0.5	0.00105	0.5
76	Germany	Neukirchen		12.96	49.25	4	2	2	0.667	0.00275	0.667
77	Italy	Milano		9.18	45.46	2	5	2	1	0.01031	1
91	**France**	**Ain (Divonne-les-Bains)**	**2016**	**6.136**	**46.3581**	**5**	**0**	**1**	**0**	**0**	**0**

Long longitude, *Lat* latitude, *n* number of sequences, *h* haplotype diversity, π nucleotide diversity. In bold, sequences obtained in our laboratory. Allelic richness after rarefaction was computed with a rarefaction size of 3

Specimens shown in bold were sequenced for this study; the other sequences have been retreived from Genbank database

Cerambycid species invasive in Europe (Pennacchio et al. 2012). Even if the two species differ in terms of bionomics and morphology, some of their characteristics overlap making identification of isolated individuals difficult, particularly at larval and egg stages (Haack et al. 2010).

Genomic DNA was extracted from adult leg or antennal fragments or small abdominal fragment from immature stages. The NucleoSpin kit (Macherey-Nagel, Düren, Germany) was used to extract DNA according to the manufacturer instructions for a final elution volume of 100 µl. DNA was amplified using the universal barcode primer pair HCO2198-LCO1490 for the COI mitochondrial gene (Folmer et al. 1994). The PCRs were done in a 25 µL total volume, with 15.8 µL ultrapure water, 2.5 µL PCR DreamTaq Green Buffer (including 20 mM of $MgCl_2$ Thermo Scientific®), 2.5 µL dNTP (10 nM), 0.5 µL magnesium chloride solution, 1 µL of each primer, 0.5 µL betaine solution and 0.2 µL Dream*Taq* DNA polymerase (5 units/µL). PCR amplifications were run on a Veriti® 96 wells fast thermal cycler (Applied Biosystems®) using the following thermal conditions: a first period at 94 °C during 5 min followed by 40 cycles of denaturation (45 s at 94 °C), hybridization (50 s at 47 °C) and elongation (1.5 min at 72 °C), and a final step of elongation of 5 min at 72 °C. PCR products were purified with a NucleoSpin gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany), following the manufacturer instructions. Sequencing reactions were made on the same thermal cycler as used for PCRs. Reactions were performed in a 20 µL volume using a Big Dye Terminator sequencing kit (v 3.0, Applied Biosystems, Foster City, CA,

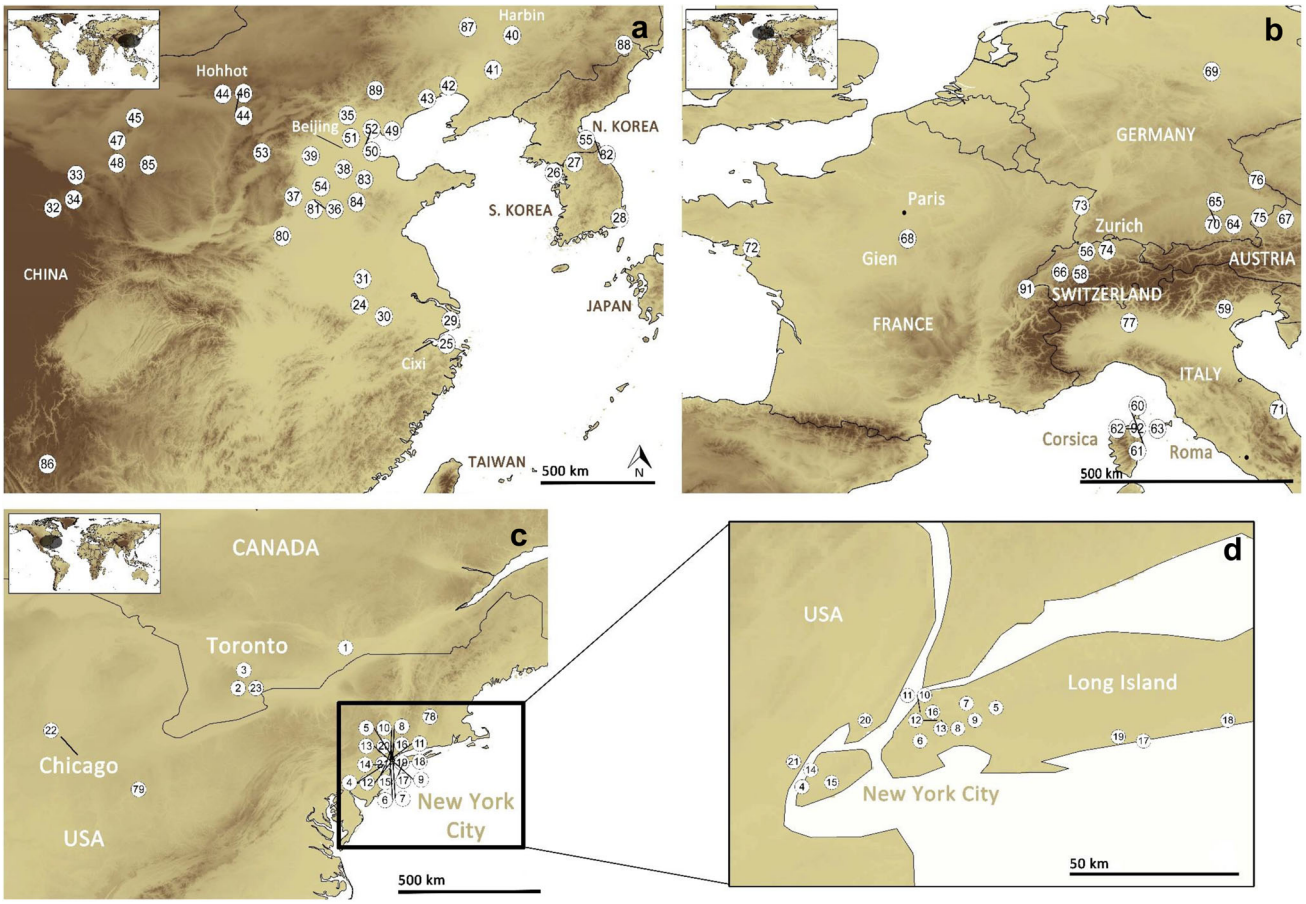


Fig. 2 Distribution of the sampling sites in Asia (**a**), Europe (**b**), North America (**c**) and around New York City (**d**). Corresponding names are shown in Table 1

USA), and both strands were sequenced in our laboratory on an ABI Prism 3500 genetic analyzer. A final fragment of 710 bp of the COI mitochondrial gene was sequenced.

Our dataset of 290 sequences was then merged with 406 sequences retrieved from GenBank (Table 1), i.e., 124 sequences from 29 Asian sites, 267 sequences from 20 North American sites and 15 sequences from 4 European sites were added, mainly resulting from the study of Carter et al. (2010). Our total dataset is thus based on 225 sequences from Asia (from 1 to 17 sequences per site), 322 from North America (from 2 to 57 sequences per site) and 149 sequences from Europe (from 2 to 17 sequences per site), encompassing 89 sites. Details of sampling and origin of the all the sequences are given in Fig. 2 and Table 1. Accession numbers of the sequences are available in Table S2.

Data analysis

In order to minimize the risk of potential pseudogenes in our dataset and to avoid overestimation of the genetic diversity, the presence of double peaks on electropherograms was checked using CodonCode Aligner V.3.7.1. (CodonCode Corporation, Centerville, MA, USA) with a specific attention on informative sites (Song et al. 2008; Haran et al. 2015). Stop codon and frame shifts were also checked using GeneDoc version 2.7 (Nicholas et al. 1997).

All the sequences were aligned using GeneDoc version 2.7 (Nicholas et al. 1997), and a final alignment of 485 bp was considered for the analysis. Common parameters of genetic diversity, i.e, number of haplotypes per site and haplotype and nucleotide diversity were calculated using DNAsp v5 (Librado and Rozas 2009). Contrib version 1.02 (Petit et al. 1998) was used to compute rarefaction to estimate the allelic richness (r) taking the sample size bias in consideration, rarefaction size being set at 2. A haplotype network was built using minimum spanning network ($\varepsilon = 0$) in PopART (http://popart.otago.ac.nz). Three main haplotypes were identified (haplotypes 1, 2 and 3), and other units (haplotypes 4–23) were colored according to their distance from the three main ones. Haplotype distribution and their frequency (Fig. 5) were projected on a map using ArcGIS 9.3 (ESRI, Redlands, CA, USA).

Analyses of molecular variance (AMOVA) were performed on Asian and European populations to measure the partitioning of genetic variance between groups of populations (Excoffier et al. 1992) in Arlequin 3.1 (Excoffier et al. 2005). A first AMOVA was performed on four clusters of Asian populations grouped by geographic proximity: beetles sampled in Western, Southern, Eastern and Central Asia, respectively. A second AMOVA was performed on geographic clusters of European populations, in order to identify a potential spread of the beetles around one or more primary introduction points. Therefore, 6 clusters were created based on geographic proximity, gathering respectively beetles from Corsica, Switzerland and nearby French sites, Southern Germany and Austria; Italy, northwest France and Northern Germany. Finally, a third AMOVA was performed on groups of European populations based on their detection date, in order to test whether populations introduced simultaneously were genetically close.

Results

A total of 23 haplotypes resulting from 23 variable sites (Fig S1) were identified out of the 696 sequences analyzed, leading to a global haplotype diversity (h) of 0.715 and a nucleotide diversity (π) of 0.00571 (Table 1). The number of haplotypes per locality ranged from 1 to 7 among the 41 native Asian sites ($h = 0.867$, $\pi = 0.00847$, $r = 0.821$), from 1 to 4 among the 25 invaded North American sites ($h = 0.715$, $\pi = 0.00603$, $r = 0.499$) and from 1 to 3 among the 23 invaded European sites ($h = 0.533$, $\pi = 0.00199$, $r = 0.548$). Three haplotypes were predominated and were common to both the native and invaded ranges (Figs. 3 and 5, haplotypes 1, 2 separated by only one mutation and, to a lesser extent, haplotype 3), representing respectively nearly 42, 29 and 12% of the total number of sequences analyzed for Europe, North America and Asia, respectively. Twelve haplotypes were private to Asia, three were private to Europe (haplotypes 5, 10 and 23, found respectively in sites 68 and 73, 76, 77 and 78) and one was private to North America (haplotype 8, site 2) (Fig. 4). Three other haplotypes were shared only between Europe and Asia (haplotypes 9, 21 and 22), and one was observed only in both North America and Asia (haplotype 4). Haplotypes common to the two invaded ranges were always also found in Asia (Fig. 4). The invaded ranges had lower allelic richness than the native range ($r = 0.548$ and $r = 0.499$ for Europe and North America, respectively, *versus* $r = 0.821$ in Asia). Less haplotype diversity ($h = 0.551$) and less nucleotide diversity ($\pi = 0.00206$) were observed in Europe than in North America ($h = 0.715$, $\pi = 0.00603$).

No clear genetic structure emerged from the Asian populations. Although the AMOVA showed that the percentage of variation was homogeneously distributed among the sources of variation (Table 2a), it revealed a slightly but significant effect of geography on the distribution of the genetic variance (23%). Hence, some haplotypes seem to be specific to their geographic areas. For example, the haplotype 4 was only found in north central China (site 44), and the haplotype 15 only in northeast China (site 33). In the same way, haplotypes 17 and 18 are predominantly found in South Korea.

Analysis of the genetic structure within Europe (AMOVA) showed that when European populations were grouped by geographic proximity, most of the variation was among populations within geographic clusters (61.54%, $p \leq 0.01$), and a relative small part of the genetic variance (24%, $p \leq 0.1$) was attributed to between geographic clusters (Table 2b). When sites were grouped by date of first detection, the highest percentage of genetic variance was attributed to variations among groups (53.59%, $p \leq 0.01$). Differences among sites within groups explained 31.41% of the variation ($p \leq 0.01$), and differences within sites were accounted for 15% of the variation ($p \leq 0.01$, Table 2c).

The two invaded ranges were each characterized by two closely related dominant haplotypes (haplotype 1 in North America and especially in Canada where it is the only haplotype sampled, haplotype 2 in Europe). The North American range also included the more distant haplotype 3, which is more frequently found in East Asia. However, this haplotype was only found in the area of New York City (Fig. 5c, d), and this area is the most diversified in North America. The genetic structure in Europe is characterized by closely related haplotypes, with the exception of one haplotype found in Milano (Italy, haplotype 3). Two haplotypes (haplotypes 2 and 10) including one that has not been found in other European sites were found in site 76 (Braunau, in Austria) where the first European infestation was detected. In France, in addition to the main haplotype 2 distributed in six sites, a private haplotype (haplotype 5) was present in central and western sites (68 and 73). In Corsica, all the sites were genetically homogeneous (haplotype 2), except for site 60 where one specimen belonged to haplotype 9, which is also found in Germany (site 69). The most diversified site in Europe was located in Germany (site 70), with three haplotypes identified.

Discussion

Since the first detection of ALB outside its native range, multiple infestations of this species have been reported across Europe and North America. The present study gives

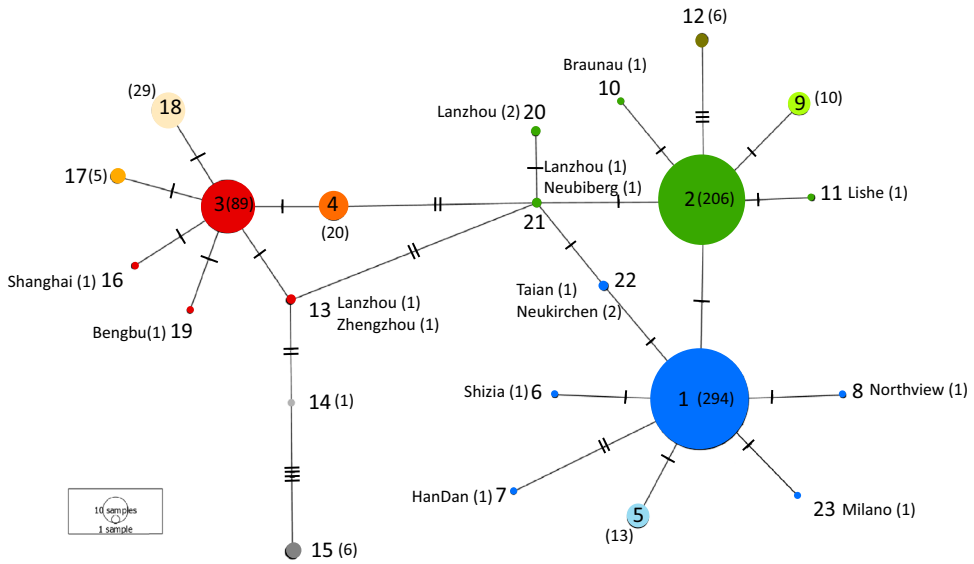


Fig. 3 Minimum spanning haplotype network. Mutational steps are symbolized by dashes, and the diameter of the circles is proportional to the number of individuals that belong to each haplotype. Numbers of specimen per haplotype are given in parentheses. Haplotype 1, 2 and 3 are considered the main haplotypes. Units gathering less than five individuals and distant less than two mutational steps from the closest main haplotype take its color. A color similar to the one of the closest haplotype is given to units gathering more than five individuals or distant of more than two mutational steps from the closest main haplotype. Finally, units that cannot connect to any main haplotype are colored in gray. We showed details for units that are not identifiable on the projection map (Fig. 4)

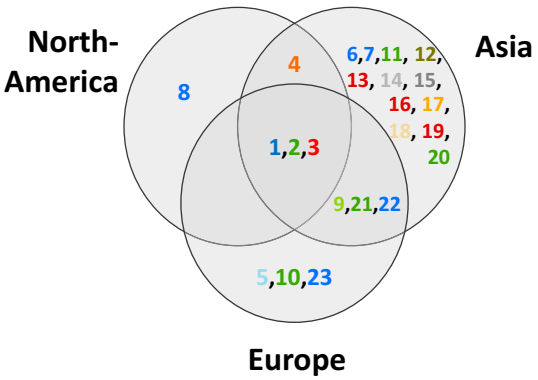


Fig. 4 Venn diagram describing the number of haplotypes that are either private, common to 2, or common to all 3 regions. Numbers and colors correspond to the haplotype network are shown in Fig. 3

an overview of the current genetic structure of the invasive populations with a specific focus on European populations. We showed that among the 23 haplotypes identified, the three most common haplotypes identified in the native range are also present in both invaded ranges, Europe and North America. Two of them are closely related and dominate all three regions, whereas the third one appears to have established only locally in New York and Milano, Italy. This provides a first step to formulate a hypothesis on the invasion pathways of this beetle across the world. Moreover, genetic analysis allowed us to confirm that all specimens sampled across Europe, including immature stages, were correctly identified as *A. glabripennis*.

Structure within the native range

As expected, the results confirm the native range of ALB as being by far the most genetically diverse area (Suarez et al. 1999; Allendorf and Lundquist 2003; Roman and Darling 2007; Yang et al. 2012), and signs of an ancestral structure may still be visible across Asia. Indeed, some haplotypes seem to be specific to their sampling areas, as shown by the analysis of molecular variance among the geographical clusters. Using RAPD, An et al. (2004) found signs of a former genetic structure in Asia. They concluded that the six Asian populations they sampled formed two clusters: the first one grouping insects from Shandong, Ningxia, Shaanxi, Hebei and Nei Mongolia, and the second comprised of specimens from Gansu. Indeed, the regions of the first cluster are quite homogeneous according to our data, with a strong abundance of the two closely related haplotypes 1 and 2. Nevertheless, we analyzed more populations from the native range, including beetles from southern areas of China, sequenced mitochondrial DNA which provides finer resolution of genetic diversity than RAPD, and generally found no clear phylogeographic structure for ALB populations in China. These preliminary results must be confirmed as the sampling was not sufficiently exhaustive, especially in southern Asia, to provide a reliable distribution of the haplotypes in Asia.

This lack of clear genetic structure is not typical of a native range (Hewitt 2000; Kerdelhué et al. 2014), which is

Table 2 Results of AMOVAs

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	*p* values
(a) AMOVA—Asian populations grouped by geographic proximity					
Among groups	3	124.828	0.58153	22.45***	0.000
Among populations within groups	36	233.108	1.03350	39.89***	0.000
Within populations	185	180.513	0.97575	37.66***	0.000
(b) AMOVA—European populations grouped by geographic proximity					
Among groups	5	46.125	0.22492	23.63*	0.079
Among populations within groups	17	60.686	0.58579	61.54***	0.000
Within populations	121	17.077	0.14113	14.83***	0.000
(c) AMOVA—European populations grouped by date of detection					
Among groups	11	86.914	0.50406	53.59**	0.007
Among populations within groups	11	19.898	0.29546	31.41***	0.000
Within populations	121	17.077	0.14113	15***	0.000

*** $p \leq 0.01$; ** $p \leq 0.05$; * $p \leq 0.1$

a: Asian sites grouped by geographic proximity, b: European sites grouped by geographic proximity; c: European sites grouped by date of first detection

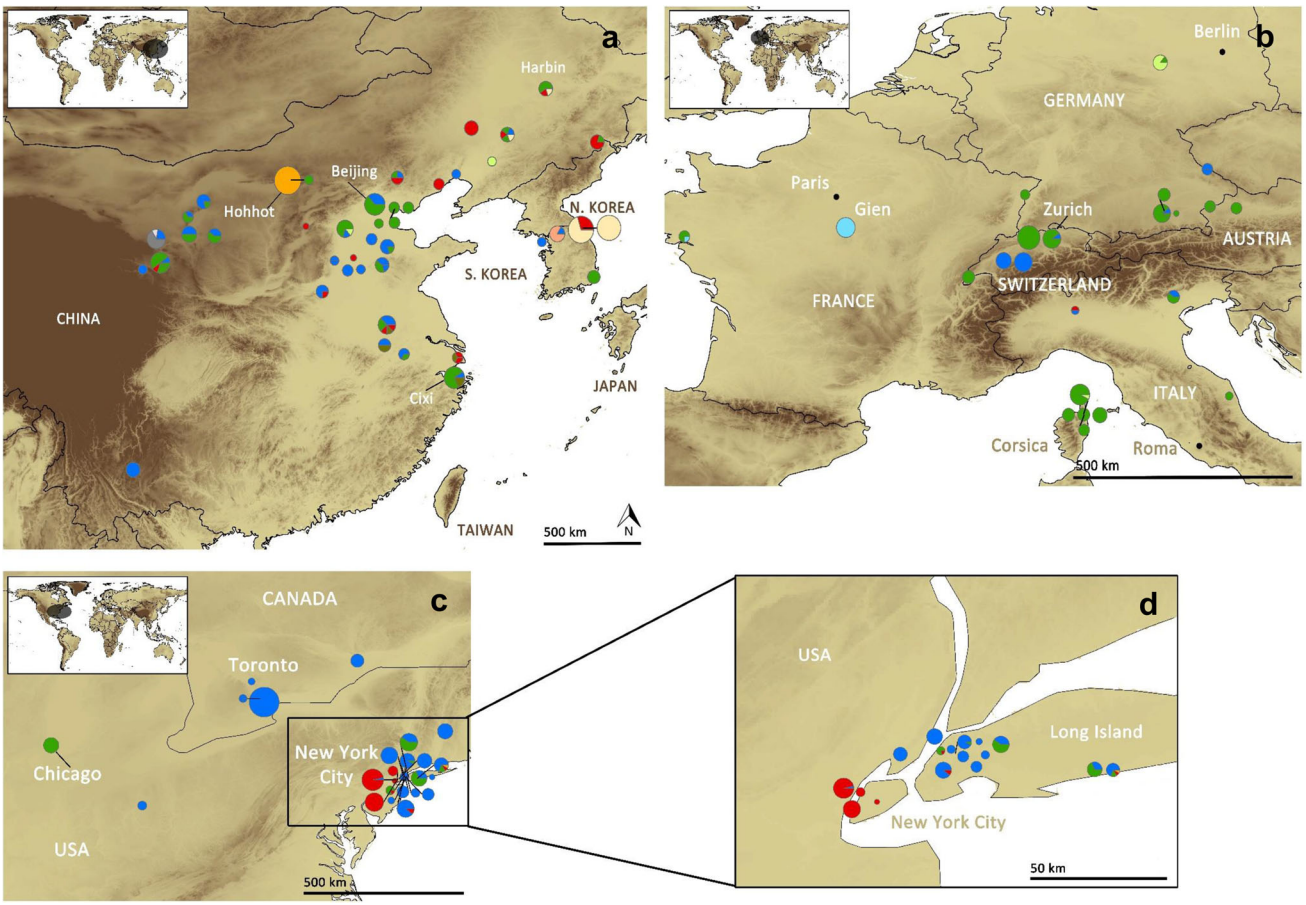


Fig. 5 Haplotype projections in Asia (**a**), Europe (**b**), North America (**c**) and the area of New York City (**d**). Colors correspond to the haplotype network are shown in Fig. 3

usually characterized by a very marked genetic identity in each geographic area. On the basis of our analyses, we hypothesize that any observable phylogeographic patterns are blurred by the human-aided movement of goods and plant material within Asia, as proposed in previous studies (Carter et al. 2009). The forest shelterbelt and the project of afforestation started in the 1980s by the Chinese government (Li 2004) may also have reinforced the human-mediated dispersal within Asia. At that time, several thousands of trees, mainly poplars, were replanted in northern and eastern China in order to reduce erosion and deforestation (Haack et al. 2010). Movement of infested trees or wood across Asia may have given rise to the observed genetic structure, and could explain the fact that northwestern sites are among the most diverse in Asia. Furthermore, the first infestation of ALB recorded in Asia dates back to the early 1980s and was located along the forest shelterbelt (Pan 2005), supporting the hypothesis of dispersal through infested trees.

Among the haplotypes sampled in Asia, the most widespread haplotypes, 1 and 2, are closely related and are found in 34 out of 41 sites. It is interesting to note that they seem especially abundant in the Hebei and Henan provinces, known for their agricultural production and thus considered as primary sites for exporting food within the country or worldwide. Similarly Cixi, West China (site 25, Zhejiang Province) is a major manufacturing location from which goods are exported and where haplotypes 1 and 2 can be found. Critical transportation corridors for goods and people into and out of these areas may have contributed to the spread of these two predominant haplotypes, both within Asia and around the world, but our dataset is not robust enough to substantiate it.

Evidence for complex invasion processes in invaded ranges

The patterns observed in Europe and North America are broadly similar. We showed that among the 23 haplotypes identified, the three most common haplotypes identified in the native range are also present in both invaded ranges. Two of them are closely related and dominate all three regions, whereas the third one had only established locally in New York City and Milano, Italy. In the two invaded continents, the distribution of ALB is probably due to several introductions followed by a secondary spread, as suggested for the North American infestations by Carter et al. (2010).

Genetic patterns in the USA seem to result from separate introductions, as well as multiple human-mediated transport events (Carter et al. 2010). Most of the populations from the USA used in this study were retrieved from GenBank (see Carter et al. 2010), and the specimens we added corroborate the invasion scenario proposed by Carter et al. (2010). Similarly in Canada, they hypothesized that the sampled population resulted from a separate introduction event. The genetic pattern observed supports the hypothesis of secondary spreads in the area of Toronto, as suggested by Turgeon et al. (2015) since identified haplotypes are identical, but could also result from multiple introductions from the same or genetically similar populations.

The presence of some distant haplotypes in Europe suggests that the current partitioning of ALB genetic diversity more likely results from multiple introductions, which is corroborated by the numerous interceptions of ALB reported (EPPO Global Database), and the history of the invasion in Europe. For instance, the first infestation was detected in Austria in 2001 (Braunau, site 75), followed two years later by the detection in the center of France (Gien, site 68). Each population is homogeneous, but genetically distinct from the other one (3 mutational steps) and therefore cannot be linked by any human-mediated dispersal. Moreover, they are too geographically distant (more than 780 km) to result from natural dispersal in a short time (Smith et al. 2001, 2004; Williams et al. 2004b; Javal et al. 2017; Lopez et al. 2017). It is therefore probable that they result from distinct introduction events. Similarly, the same haplotype (haplotype 5) is found in Gien and Sainte-Anne-sur-Brivet (respectively, site 68 and 72), two French sites more than 300 km apart where ALB was detected, respectively, in 2003 and 2004. In Gien, ALB has probably been introduced via wood packing material imported by a private company located in the city. In Sainte-Anne-sur-Brivet, the beetle is suspected to have arrived with packed stones used for public work (Hérard et al. 2006). Apparently, the stones were the only material packed in wood, and this work did not include import of ornamental plants that could also have been a vector for the beetle (Haack et al. 2010). All the beetles responsible for the infestation in Sainte-Anne-sur-Brivet likely arrived simultaneously, and this introduction event was probably distinct from the one in Gien. Moreover, it appears that within even a small geographical area, populations are genetically different (Table 2b) which corroborate the hypothesis of multiple introductions or a single introduction with material from various source populations. Similarly, comparison of European populations on the basis of the date of their first detection showed that from one year to the next, the introduced populations are genetically different, suggesting that the European infestations may be due to several introduction events, either from different sources, or from the same source but with different haplotypes surviving the bottleneck effect. These results should be further validated since it may take several years before an infestation is detected, and initial establishment

times are unknown but only inferred based on dendrochronology of oviposition pits and exit holes, so do not reflect the precise invasion history of the species (Favaro et al. 2013).

Considering the globally observed diversity and genetic structure, the hypothesis of several introduction events followed, at least for some locations, by secondary spread is realistic for both invaded areas, especially considering the fact that both the dominant haplotypes in Europe and North America are quite abundant in the native range. The observed pattern and especially the predominance of some closely related haplotypes could be due to closely related founders. A bridgehead scenario cannot be ruled out between the two invaded areas (Lombaert et al. 2010; Yang et al. 2012). Under such a hypothesis, beetles might have infested locations in North America first (based on estimated initial infestation times in the US predating these in Europe), and then have been shipped to Europe via wood packing material or wood logs prior to the current wood treatment regulations being put in place (Haack et al. 2010). However, on the basis of our data, we can neither determine which haplotype might have been involved in this potential bridgehead scenario, nor can we identify a source population in North America and an arrival site in Europe for such a scenario.

Human-mediated versus natural dispersal within invaded range

It is clear that the first introductions of ALB into new continents are due to passive human-mediated transportation. However, the sheer number of establishments found across both North America and Europe raises the question of the relative importance of multiple introductions from overseas, as opposed to within region human-mediated transportation and active natural dispersal (i.e., secondary spread). Multiple transport events of ALB are suspected to have occurred within the invaded North American region (Carter et al. 2010) and are assumed for the native range as well (Carter et al. 2009). Anthropic dispersal has also occurred within Europe, since beetles from Marly (Switzerland, site 66) were transported through firewood to Brünisried (Switzerland, site 58) where they contributed to the formation of another infestation (Eidg. Forschungsanstalt WSL 2014). In Austria and the south of Germany as well as in Corsica, the sites sampled are geographically very close and genetically identical or distant by only one mutational step. Human-mediated dispersal could be at the origin of these patterns, but the actual flight capacity of ALB should be taken into account in these specific cases. The beetle is known to be a bad disperser that tends to stay on the same tree when the resource is sufficient (Smith et al. 2004; Williams et al. 2004b; Sawyer 2009). However, initial bottlenecks in invaded sites may have selected individuals with some specific behavioral characteristics or host preferences (Estoup et al. 2016) possibly due to their primary origin, and the reduced population density linked with the new characteristics of the invaded ecosystem could lead to different behavioral patterns in the introduced individuals (Trotter and Hull-Sanders 2015). Furthermore, estimates from models suggest that in some rare cases beetles could disperse longer distances from their emergence tree (Trotter and Hull-Sanders 2015). The beetle's ability to disperse over longer distances has been confirmed by recent standardized measurements of dispersal capacities on flight mills that showed ALB could fly up to 14 km over its lifespan (Javal et al. 2017; Lopez et al. 2017). However, none of the infestations in invaded areas has expanded rapidly over a large geographic area to date, and all the invasive populations have stayed in the urban or peri-urban environment. ALB adults have the ability to disperse, but longer distance dispersal remains a very rare event.

To conclude on this point, even if it is not possible to tell natural from anthropic dispersal, our results suggest that in some cases, once introduced some populations undergo a secondary spread. This is supported by the results of the AMOVA performed on geographically regrouped populations that showed differences between groups were significant but slight. This tendency may be due to rare supposed and observed secondary spread of introduced populations close to their introduction site.

Conclusion and perspectives

This work gives an overview of the genetic structure of ALB in its invaded areas and reveals a presumably complex process of invasion, potentially combining multiple introductions in both native and invaded ranges, followed by secondary spread via anthropic or natural means. The multiple introduction pattern suspected for ALB is common for IAS (Roman and Darling 2007; Dlugosch and Parker 2008). An ever increasing number of examples are found in the literature, revealing that most of the invasion processes include very complex scenarios (Estoup and Guillemaud 2010; Garnas et al. 2016) that not only deeply influence the genetic structure of the invasive populations, but also their ability to thrive in their new environment (Dlugosch and Parker 2008). Therefore, the success of ALB settlement in Europe could be due to the potential beneficial effect that multiple introductions could have had on its populations. The invasion scenario hypothesized in this study may also have implications in terms of prevention and management of the invasion. The multiple introductions combined with the secondary spread indeed highlight the fact that effort should not only be made at

eradicating the established populations, but also at preventing new introductions at points of entry and limiting human-mediated spread of the established populations.

Finally, higher resolution DNA markers, such as microsatellites, together with methods for inference of invasion pathways (Guillemaud et al. 2010; Pudlo et al. 2015) would give more accurate information on the dynamics of the introductions and help to better understand the global biogeography of the invasive populations at different scales. With a finer scale information, it may be possible to determine the origins of the infestations in the invaded areas, as well as how the ALB has secondarily spread to create new infestations. This may bring to light some additional human-aided pathways that could be shut to prevent additional secondary spread.

Authors' contributions

MJ, GR and AR designed research. MJ conducted experiments. MJ analyzed data with JH and GR. MK, FH and AR gave expertise on species; GR and JH on method. MJ wrote the paper. All authors approved the final version.

Acknowledgements We are grateful to Marie-Claude Bon and Lincoln Smith for comments on the manuscript, and we thank Camille Bagnis, Brigitte Delahaye-Panchout, Christine Daviet, Massimo Faccoli, Jianting Fan, Gernot Hoch, Stephan Koenig, Raphaelle Moutet, Simone Prospero, Rémi Rossignol, Jing Tao, Jean Turgeon, Beat Wermelinger, Jake Wickham and Shixiang Zong for providing information and samples for analysis. We greatfully acknowledge financial support from the French Ministry of Agriculture, Food, and Forestry (Convention DGAL E07/2014 "Estimation de l'impact écologique du Capricorne Asiatique *Anoplophora glabripennis* sur les populations xylophages natives et leurs organismes associés, et traçage génétique de l'origine de ses populations invasives en France continentale et en Corse"), and from the Région Centre Val de Loire. We also thank three anonymous reviewers for helpful comments on the manuscript.

Compliance with ethical standards

Conflict of interests None of the authors declare conflict of interest for the present study. Specimens sampled did not involve endangered nor protected species.

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