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Tracing the origin of the alien pest *Cydia pomonella* in Algeria through a worldwide comparison of the species' DNA barcodes

Reserche de l'origine du ravageur exotique *Cydia pomonella* en Algérie grâce à une comparaison mondiale des codes-barres ADN de l'espèce

Rastreando el origen de la plaga exótica *Cydia pomonella* en Argelia mediante una comparación mundial de los códigos de barras del ADN de la especie

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Abstract

1. DNA barcodes (i.e., gene sequences used as identifiers for species identification) constitute a very useful tool in invasive pest research. Using them, we studied, for the first time, the origin and population structure of the alien *Cydia pomonella* in Africa, a major apple pest.
2. We sequenced a fragment of the mitochondrial gene Cytochrome c oxidase subunit I (COI) from *C. pomonella* caterpillars collected in northern Algeria and compared them with DNA barcodes from the rest of the world available at public repositories.
3. The phylogeny built upon that COI database supports the European origin of the species: haplotypes at the base of the phylogeny were registered in this continent. In Algeria, the results suggest that this pest could have been introduced from

Europe, with two unique African mutations probably favoured by *C. pomonella* multivoltinism and large population sizes.

4. Population genetic analyses at three Algerian localities showed neither structure nor founder effects. However, to confirm the underlying process of the observed population patterns, it is necessary to perform analyses using genes with higher mutation rates.
5. Compared to higher resolution markers, mitochondrial DNA barcodes are still a cost-effective tool for taxonomic determination of unknown specimens and, sometimes, may also allow a preliminary tracing of its origin. This is crucial to detect and stop the spread of invasive pests (i.e., borders). We encourage further studies including functional genes to assess whether any mutations are promoting *C. pomonella* adaptation to the Northern Africa environment.

KEYWORDS

apple pests, codling moth, *Cydia pomonella*, DNA barcoding, invasive pests, molecular species diagnosis

INTRODUCTION

The appearance of DNA barcoding almost two decades ago revolutionized a high number of basic and applied biological disciplines, including pest management science (Jinbo et al., 2011). DNA barcoding provided a new way of rapid species identification using certain gene sequences as molecular species-specific identifiers (Hebert et al., 2003). For instance, it allows a rapid molecular diagnosis of species identity, especially in the case of certain life stages (e.g., larvae) for which sometimes there are not reliable morphologically based identification keys (Ahrens et al., 2013; Amano & Higo, 2015; Bonal et al., 2011). In the case of invasive pests this is very important, since DNA barcoding may additionally show the geographical origin of the invasive individuals as mitochondrial haplotypes are usually not homogeneously distributed throughout the species distribution range (Bonal et al., 2018; Li et al., 2015). In this study we DNA barcoded, for the first time, larvae of the invasive *Cydia pomonella* L. (*Lepidoptera*) collected in Algeria and investigated the possible origin of this pest, which was recorded for the first time in Algeria by Delassus et al. in 1930, and confirmed by Frezal in 1939.

The codling moth *C. pomonella* (L.) (*Lepidoptera*: *Tortricidae*) is a harmful fruit pest that provokes dramatic economic losses worldwide (Mansour, 2019; Tian et al., 2016). In northern Algeria, apple production has a great socioeconomic importance, and the damage caused by *C. pomonella* may affect 45% of the crop and scale up to 93% if the orchard is not treated (Belkadi & Hamli, 1998; Guermah & Medjdoub-Bensaad, 2016). Moth larvae leave the apple mesocarp drilled by galleries and unavailable for commercial sale. The percentage of apples infested by *C. pomonella* usually stays above the thresholds recommended for intervention (Chen & Dorn, 2010; Pajac et al., 2011; Wan et al., 2019). The species is native to Europe, but now is present in apple orchards throughout the globe (Franck et al., 2007; Pajac et al., 2011; Wearing et al., 2001). The transport of apples and apple trees to other continents by Europeans aided the spread of the codling moth, being now found in America (Jones et al., 2013), Australia (Il'ichev, 2004) and Africa (Mahi et al., 2021).

The economic importance of *C. pomonella* has boosted the number of studies on different aspects of its biology, including its genetic characterization at different spatial levels. Many of them used fragments of mitochondrial genes (e.g., Li et al., 2015; Meraner et al., 2008) or nuclear markers like DNA microsatellites (single sequence repeats [SSR]) (Cichón et al., 2021). In recent years, the development of next generation sequencing has been a tremendous step forward and today, not only the full *C. pomonella* mitogenome is available (Shi et al., 2013), but also large chromosome assemblies to analyse the function of specific genes (Wan et al., 2019).

C. pomonella genetics is thus not an unexplored field, however, the geographical coverage of the data is largely unbalanced. Whereas detailed studies on the species population genetics exist for different areas of Europe (Meraner et al., 2008), America (Cichón et al., 2021) and Asia (Li et al., 2015), there is a total lack of data for other continents, like Africa. For instance, no DNA barcodes from Africa exist in the Barcode of Life Datasystem (BOLD), the world's largest database that registers all the barcodes published and either directly uploaded or mined from GenBank (Ratnasingham & Hebert, 2007).

The absence of African DNA barcodes is striking, since records of invasive established populations of *C. pomonella* in this continent date back at least to the early 1900s (see Wan et al., 2019). Moreover, apple production is an important economic income in certain areas of the continent, where the climatic conditions allow apple tree growth (Southern and Northern Africa) (Mahi et al., 2021; Timm et al., 2008). In Northern Africa, apple orchards are present in the provinces close to the Mediterranean coast of Morocco, Algeria and Tunisia.

We carried out this study in Algeria with the aim of sequencing, for the first time, African *C. pomonella* individuals and assessing their genetic relationship with haplotypes recorded elsewhere in the world. We retrieved the standard fragment of the mitochondrial gene Cytochrome c oxidase subunit I (COI) (Folmer et al., 1994) to assess the following questions: (i) what is the intraspecific phylogenetic relationship of Algerian *C. pomonella* haplotypes with the rest of DNA barcodes publicly available; (ii) what can these relationships tell us about the origin of the invasive populations of this pest; (iii) to what extent is there gene-flow at a regional scale among Algerian populations of *C. pomonella*.

MATERIALS AND METHODS

DNA extraction and sequencing

We collected a total of 40 *C. pomonella* larvae from three different North Algerian apple orchards: Sehala Thaoura (35°12′09″N, 0°50′07″W; 14 individuals), Sidi Lahcen (35°08′17″N, 0°41′00″W; 13 indiv.) and Tenira (35°00′27″N, 0°35′25″W; 13 indiv.). The maximum pairwise distance between sampling sites was 50 km. *C. pomonella* caterpillars were collected at cardboard strips placed around the trunks. The trap strips were installed from the beginning of July (at the end of the first generation). In accordance with the protocol recommended by Agricultural Technical Coordination Association (1974), 30 trap strips were set up per apple orchard, at the rate of one trap per tree. Trap strips are 25 cm wide and 30 cm long. All caterpillars were preserved upon collection in 96% alcohol and taken to the laboratory. DNA was extracted from a piece of caterpillar tissue (2 mm) using commercial extraction kit (EZNA® Tissue DNA Kit) according to manufacturer's instructions. No negative controls were used in the extraction, but they were used in the Polymerase Chain Reaction (PCRs) routinely (see below).

We amplified two fragments of the mitochondrial gene COI of each individual. The first fragment was amplified using the standard primer pair for DNA barcoding LCO1490/HCO2198 (Folmer et al., 1994). For the second (herein Pat & Jerry's COI), we used the primers C1-J-2183 (Jerry) and L2-N-3014 (Pat) (primer and PCR details at Hughes & Vogler, 2004). These two fragments do not overlap at all (Ouvrard et al., 2016) and in both of them we used identical gene amplification routines. Reaction volume was 15 μl (14 μl Mastermix +1 μl DNA). We used IMMOLASE™, a heat-activated thermostable DNA polymerase (Bioline). PCR cycling regimes were the following: a 9 min denaturation at 95°C, 40 cycles of 30 s denaturation at 94°C, a 45 s annealing at 50°C and 45 s min elongation at 72°C, with a final extension step of 10 min at 72°C. PCR success was verified in a gel electrophoresis, in which negative controls were included; we never retrieved bands in the negative controls, and we could thus disregard any potential for contamination. PCR products were sent to Macrogen Inc. (Madrid, Spain's delegation), and went through a direct sequencing after purification. Sequencing was performed using Big-Dye (Perkin-Elmer) technology and an ABI3700 sequencer. We assembled, inspected and edited the chromatograms using Sequencer 4.6 (Gene Codes Corp., Ann Arbor, MI, USA), obtaining good quality DNA sequences from 37 individuals for the Folmer region of the COI gene and from 39 individuals in the case of the Pat & Jerry fragment. The first region of the COI gene (Folmer) was used for the worldwide comparisons of *C. pomonella* sequences, whereas the second (Pat&Jerry) was added to the first (Folmer) to get a longer sequence to be used for intra-specific population genetic analyses within Algeria. The sequences of these fragments were trimmed to 620 and 790 base pairs, respectively, to reduce the proportion of missing data. We did so because phylogenetic inference software does not consider those DNA positions with missing data, and this is what may happen if some sequences are shorter and produce a matrix with terminal gaps. We translated all the sequences into amino acids using the software MacClade (Maddison & Maddison, 2005) to make sure that there were not nuclear mitochondrial insertions (numts) or stop codons (as mitochondrial DNA has to be intronless) (see Bonal et al., 2018).

Molecular analyses

DNA database alignment

We searched for all the DNA barcodes available for *C. pomonella* in the BOLD (Ratnasingham & Hebert, 2007). Its search engine (https://www.boldsystems.org/) contains the largest database of DNA barcodes in the world; it includes all those uploaded directly by researchers to this base and those sequences submitted to GenBank and later mined by BOLD.

A total of 284 DNA barcodes were downloaded from BOLD, including sequences from Europe, America, Australia and Asia. Some of them corresponded to specimens intercepted at US borders, which were used to be compared against those of known collection site to try to investigate their potential origin. All sequences from BOLD were pooled with the DNA barcodes from Algeria (the Folmer region of the COI gene) sequenced by us. We aligned all together using MUSCLE software (Edgar, 2004) as implemented in MEGA 7 (Kumar et al., 2016; default values). We discarded all those sequences shorter than 600 base pairs and those with a low overlap (lower than 85%, see Table S1 for the Accession numbers of the sequenced maintained). The sequences kept for further analyses were pooled with those from our study sites in Algeria to get a final database of 176 sequences, which were collapsed into unique haplotypes using the online .fasta sequence toolbox FaBox (Villesen, 2007).

Phylogenetic reconstruction and evolutionary relationships between haplotypes

We assessed the phylogenetic relationships among the available *C. pomonella* haplotypes for the Folmer region of the COI gene using Bayesian inference analyses as implemented in Mr Bayes 3.2 software (Ronquist et al., 2012). To do so, we selected one sequence of each haplotype and a sequence of another species of the same genus (*Cydia fagiglandana*) as outgroup. The nucleotide substitution models used in the phylogenetic reconstruction was GTR + I + Gamma. We calculated the substitution models for each of the three codon positions with PartitionFinder version 1.1.1 (Lanfear et al., 2012).

The parameters for the Bayesian inference were set up to two parallel runs of 5 million generations each conducted using one cold and two incrementally heated Markov chains ($\Lambda = 0.2$), sampling every 5000 steps. We reviewed the standard convergence diagnostics implemented in Mr Bayes and the average standard deviation of the split frequencies to assess that the Markov chain had reached stationarity. After 1,250,000 generations, the average standard deviation of the split frequencies stabilized in values close to zero (0.001) and the phylogenetic trees were summarized using the all-compatible consensus command with 25% burn-in.

In addition to the phylogenetic trees, we used POPART software (Leigh & Bryant, 2015) to build statistical parsimony networks (Templeton et al., 1992) among all the distinct haplotypes of *C. pomonella* to assess their evolutionary relationships. Doing this we could also assess sequence mutations along their spread over the world from their native European range.

Population genetics of *C. pomonella* populations in northern Africa

We calculated the basic population genetic parameters for the three sampling sites in Algeria, namely gene diversity (H) and nucleotide diversity (π). We also tested for signals of recent local population bottlenecks or expansions by means of Tajima's D (Tajima, 1989) and Fu's FS tests (Fu, 1997). We further assessed the existence of genetic structure among the three populations with analyses of molecular variance (AMOVAs) as implemented in ARLEQUIN version 3.0 (Excoffier & Schneider, 2005). For all the population genetic analyses with Algerian samples, we not only used the Folmer region of the COI gene of Cytochrome oxidase I, but added the Pat & Jerry's COI fragment to get a final sequence of 1410 base pairs for each individual.

RESULTS

In total (joining the sequences from our study sites and those downloaded from the BOLD), we retrieved 34 distinct haplotypes. We removed two of them (downloaded from BOLD) because of their large genetic divergence compared to the other sequences, probably as a consequence of an incorrect taxonomic identification. In the African (Algerian) samples, we got good quality sequences from the Folmer region of the COI gene for 90% of the samples (36 of the 40 sequences); the remaining 10% were discarded and not used in further analyses. Good quality sequences had well-resolved peaks that could be told apart from adjacent ones and no double peaks. We retrieved a total of seven distinct haplotypes (GenBank Accession numbers PQ07322 to PQ07328), two of them were exclusive for Algeria and not found in any other of the 140 sequences (from America, Europe, Australia or Asia) downloaded from the BOLD (Table 1). The most abundant haplotype in Algeria (number 10) is also very abundant in other continents (Europe and America) and has also been recorded in the caterpillars intercepted in the Quarantines of the Unites States border (Table 1 and Figure 1). Other common haplotypes in Africa/Algeria were also in Australia, America and Europe (e.g., numbers 7 or 20) (Table 1 and Figure 1). This shows that the most widespread haplotypes in Algeria are quite cosmopolitan.

The haplotypes exclusive to Africa/Algeria were retrieved from just one individual and were closely related (only one mutation) from the most common haplotype (Figure 1). The presence of exclusive haplotypes also occurred in Europe, America and Australia (Table 1). They were mostly found in one or two individuals and also closely related (only one mutation) to common haplotypes in each of those continents. The only exception was Asia, as the 30 samples collected in India corresponded to the same haplotype (Figure 1). Europe had the highest number of haplotypes regarding the number of sequences. Moreover, leaving apart the exception of India, Europe also showed the highest percentage of exclusive haplotypes (53%). These European haplotypes also occupied a basal position in the phylogenetic tree (Figure 2); all of them had been collected in this continent (exclusively or in Europe and elsewhere). The phylogenetic tree showed two distinct clusters (strongest support, PP = 1, haplotype 22 at the base of the second), but in both, there were haplotypes from Europe (the hypothetical origin of *C. pomonella*), and from continents in which it has been introduced (America, Africa and Australia).

The analyses at the population level carried out only with the Algerian samples showed that there was a great genetic similarity among the three localities. Population structure analyses (AMOVA) were not significant ($p = 0.78$). There was no genetic differentiation between sampling sites, all the genetic variability was explained at the "within population" level (Table 2). The number of distinct haplotypes retrieved from the sequences containing the two fragments of the COI gene was 11, but most of them were shared among localities (Table 3). One of the localities (Tenira) had the highest number of haplotypes and also the highest gene diversity (0.92; Table 4); nucleotide diversity was similar among the three sites. All neutrality tests (Tajima's D and Fu's tests) showed that neither of the three populations departed from equilibrium significantly.

DISCUSSION

Globalization has increased the international movement of plant material along with the risk of alien pest introduction, which constitutes a major environmental and economic problem (El Sheikha, 2019; Tian et al., 2016). Alien species are not always introduced as adult insects, but frequently as eggs, larvae and/or pupae, for which morphological identification keys are frequently lacking. In this context, molecular determination has become an extremely useful tool to readily determine alien species upon arrival (El Sheikha, 2019). Using DNA barcoding, we identified the alien pest *C. pomonella* from caterpillars collected at three apple growing regions of Algeria, which were also the first ever sequenced in Africa to our knowledge.

TABLE 1 Upper: number of samples of each haplotype (27 and 29 excluded) at each continent and intercepted at borders (Quarantine); and lower: ratios between the numbers of haplotypes and total sequences and between exclusive haplotypes and total haplotypes at each continent.

Haplotype	Africa	America	Asia	Australia	Europe	Quarantine
1				**2**		
2					**2**	
3						1
4		3		1		
5		**1**				
6			**30**			
7	7	6		4		
8					**2**	
9		**1**				
10	12	6			5	5
11						1
12						1
13		9		3	1	
14						1
15					**1**	1
16		3			1	
17					**2**	
18	**1**					
19	**1**					
20	9	6			4	2
21						4
22	3				6	
23					**1**	
24		**1**				
25				2	1	3
26					**1**	
28		**1**				
30					**1**	
31	3			3	2	1
32						1
33				**4**		
34					**1**	
Number of sequences	36	37	30	19	31	21
Number of exclusive haplotypes	2	4	1	2	8	
Number of haplotypes	7	10	1	7	15	
Ratio haplotypes/sequences	0.19	0.27	0.03	0.37	0.48	
Ratio exclus. haplo/tot. haplo.	0.29	0.40	1.00	0.29	0.53	

Note: Bold figures correspond to haplotypes only recorded at one continent.

Despite the increasing number of molecular markers/techniques, DNA barcodes, which started to be commonly used more than 20 years ago (Hebert et al., 2003), are still very valid for early species detection (El Sheikha, 2019). Molecular detection of invasive species is increasingly being used around the world due to the accessibility of equipment and training in a growing number of institutions worldwide. The growing number of sequences available at public repositories (such as GenBank or the BOLD) (Ratnasingham & Hebert, 2007) facilitates the identification of the query sequences to the species or a coarser taxonomic rank.

We compared the *C. pomonella* DNA barcodes sequenced in this study with all those available at public repositories in order to reconstruct its probable invasion from Europe to Africa. From the seven haplotypes retrieved in Algeria, all of them (except two single-

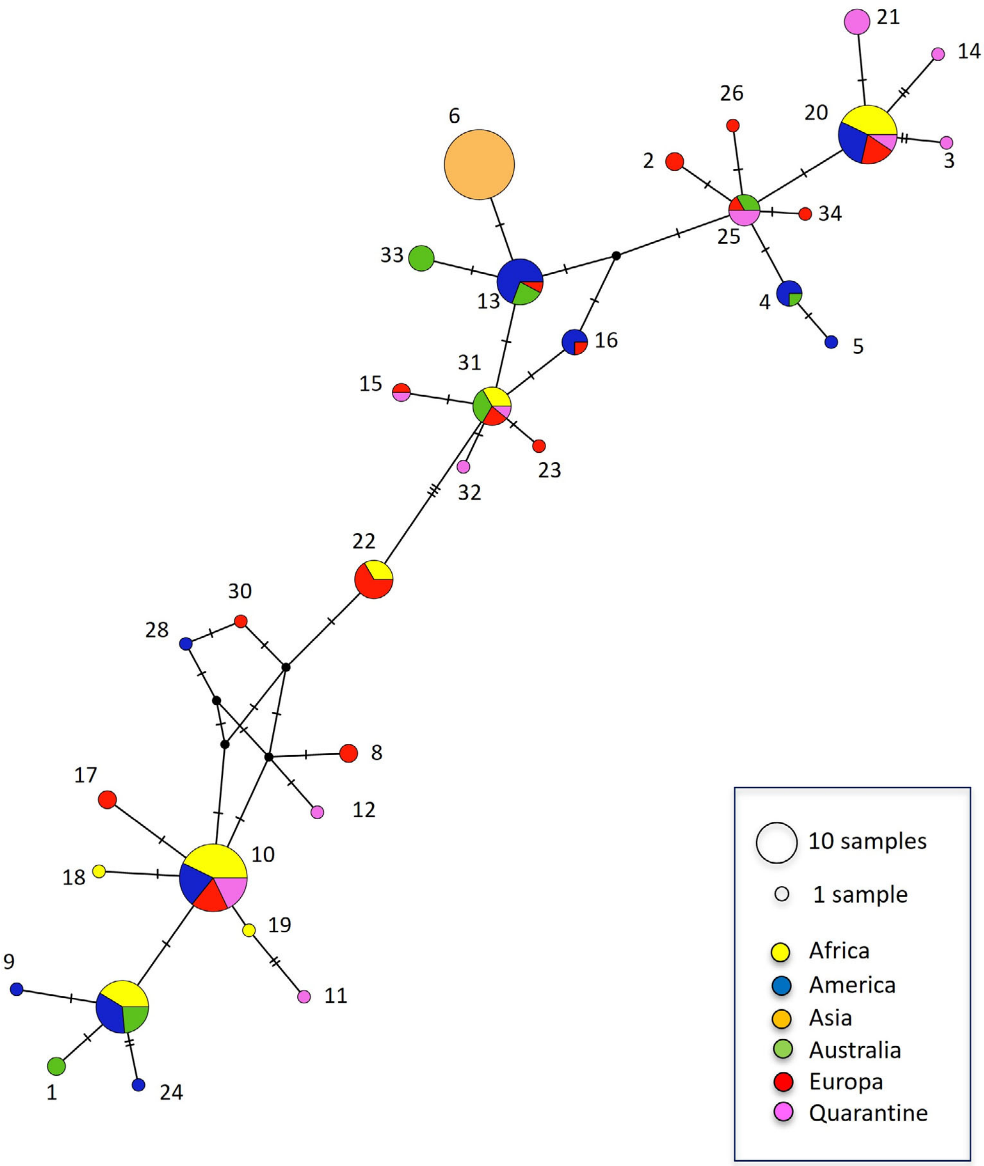

FIGURE 1 Statistical parsimony networks of all mtDNA haplotypes (Folmer region of the COI gene, 620 base pairs) of *Cydia pomonella*. Each circle represents a different haplotype (haplotype number shown besides). The size of the circle is proportional to the number of individuals possessing it, with colours denoting the proportional representation of sample sources. The dashes crossing the connection lines show the number of mutations among haplotypes. The colours correspond accordingly: yellow (Africa/Algeria), blue (America), orange (Asia/India), green (Australia), red (Europe) and pink (Quarantine, individuals intercepted at US borders).

individual ones) are found in different continents; in all cases except one Europe is among them (including the two most widespread ones, H10 and H20, see Figure 1). The proximity of the European Mediterranean coast and a long history of agricultural products interchange, and human migration, especially between Algeria and France (Sessions, 2011), probably explains this haplotype's likely origin. As an example of the spread of agricultural pests from France to Algeria, Ryan et al. (2019) indicated that the small cabbage white butterfly, *Pieris rapae* (Lepidoptera: Pieridae) had been introduced (200 B.P.) to Algeria during the French colonial period. In spite of the preceding results, we have to be cautious about making any statement on invasion routes, as DNA barcodes are not as powerful as newer and more expensive techniques (e.g., RAD-seq high-throughput Sequencing Data) employed in these tasks (Ortego et al., 2023). Nonetheless, the less costly DNA barcodes can be used to identify the origin of invasive organisms, and still be accurate, when their distribution at the native range is clearly segregated (e.g., Li et al., 2010; Ramos et al., 2023).

Pest border controls are one of the more direct applications in which DNA barcoding has significantly advanced monitoring of insects. DNA barcoding allows a quick and reliable diagnosis of introduced pests, even when only a little bit of the tissue is found. The comparison with other sequences of the same gene may also permit to assess the origin, which is essential to block certain invasion routes (Amano & Higo, 2015; Bonal et al., 2018; El Sheikha, 2019). In the

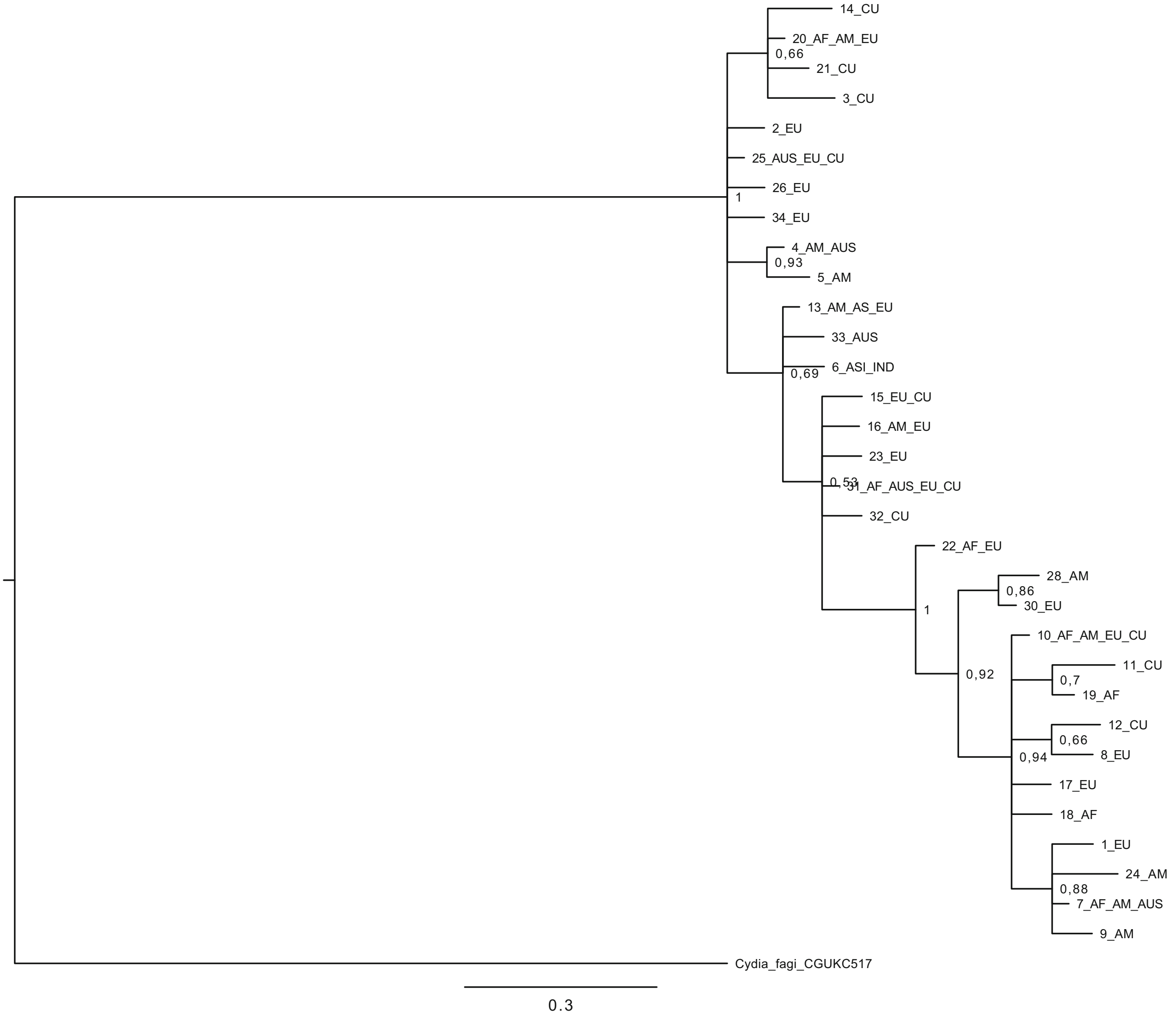

FIGURE 2 DNA phylogeny of all mtDNA haplotypes (Folmer region of the COI gene, 620 base pairs) of *Cydia pomonella*. Tree topology was inferred Bayesian inference (GTR + I + Gamma substitution model). Support for each node is represented by the Bayesian probability value (below the branch). The congeneric species *Cydia fagiglandana* was used as an outgroup. Each branch terminal corresponds to a unique haplotype. The label shows the haplotype number and the initials of the place where it was recorded: Africa (AF), America (AM), Australia (AUS), Europa (EU) or intercepted at US borders-quarantine (CU).

case of *C. pomonella*, we tried to identify the origin of insects intercepted in US borders (pink colour in Figure 1) comparing them with our samples and the sequences available at the BOLD (Ratnasingham & Hebert, 2007. Figure 1). However, due to the cosmopolitan distribution of this pest, we could not identify their origin undoubtedly, and so our attempt to investigate potential invasion routes was relatively unsuccessful. Two haplotypes sequenced in US borders (H10 and H20) were found in Africa, but also in other continents. Moreover, there were a few single haplotypes only retrieved at the border controls (e.g., H3, H32, see Figure 1), and so no geographical links could be established.

Previous phylogeographic studies in European populations based on mitochondrial DNA have revealed the existence of two clades (Meraner et al., 2008). Our study, which includes sequences from all over the world, has retrieved them in the Bayesian tree and, more clearly, in the phylogenetic network (Figure 1 and Figure 2). In the phylogenetic tree (Figure 2), haplotype 22 would be at the base of the second clade. In the network, the connection between this haplotype and haplotype 31 is the one that accumulates more mutations, depicting the gap between the two groups (Figure 1). In Europe, the causes underlying the split could be climatic, and could have provoked the temporal division of the species distribution range between the lower and middle Pleistocene, about 1.29–0.20 million years ago (Meraner et al., 2008). These two main clades mixed again after further population expansion in Eurasia. Our gene tree, which adds sequences recorded from non-European localities, shows that

TABLE 2 Results of the intraspecific AMOVA analyses showing the partition of the total genetic variance between local populations.

Source of variation	df	Sum of squares	Percentage of variation
Among populations	2	4.14	0
Within populations	32	163.77	100

TABLE 3 Number of samples of each haplotype at each sampling locality of Africa (northern Algeria).

Haplotypes	Sehala Thoura	Sidi lahcen	Tenira
1	3	5	3
2	4	2	2
3			1
4			1
5	1		1
6	1		
7	1		
8			1
9	2	4	1
10		1	
11			1

TABLE 4 Summary of the genetic information for each African (northern Algeria) sampling locality.

	Sehalathoura	Sidi lahcen	Tenira
Number of individuals	12	11	11
Number of haplotipes	6	4	8
Gene diversity	0.84 ± 0.07	0.74 ± 0.08	0.92 ± 0.06
Nucleotide diversity	0.0071 ± 0.003	0.0076 ± 0.004	0.0069 ± 0.003
Tajima's D	1.41 ($p = 0.94$)	2.42 ($p = 0.99$)	0.50 ($p = 0.72$)
Fu's F	3.16 ($p = 0.92$)	7.15 ($p = 0.99$)	0.22 ($p = 0.54$)

Note: The table includes the total number of individuals sequenced, the number of haplotypes, gene and nucleotide diversity (mean ± SE) and the results and associated probability of the neutrality tests (Tajima's D and Fu's F).

nowadays both of them occur in all the continents where the pest is present. The two main haplotypes of the two groups (H10 and H20; Figure 1) are present in Africa, Europe and America, for example. The continuous exports of apples and apple trees from Europe (pest native region) to the rest of the world (Codron et al., 2019) has probably favoured this widespread distribution of haplotypes from different clades, which have even reached countries of the Far East like China (Li et al., 2015).

Despite the exact origin of *C. pomonella* remaining disputed between South East Europe and Asia Minor (see Wan et al., 2019), Europe is likely the centre from which the pest spread worldwide. In evolutionary terms, it is still early for the new invasive populations to evolve independently to a great extent, especially in the case of mitochondrial DNA variants. However, in some cases, there are single haplotypes found only in invaded areas, which might have originated locally. The network shows that they are most closely related to major haplotypes present in the area (Figure 1), which is typical of expanding populations (Milá et al., 2000; Teixeira et al., 2011). One example is Africa, with two unique haplotypes (18 and 19) originating from haplotype 10, the most common in Algerian populations (Figure 1). The same happens with haplotypes 9 and 24 in the case of America (Figure 1).

The appearance of new haplotypes takes time, as it depends on mutation rates, but it may accelerate when reproductive rates are high. This is the case for insects, like *C. pomonella*, which in northern Africa may have up to four generations each year (Hmimina & El Iraqui, 2015; Soltanin et al., 1986). Relatively high rates of appearance of new haplotypes (time scale of a few years) have been previously recorded in organisms subjected to quick population growth (Teixeira et al., 2011). Following this argument, new haplotypes in newly invaded areas should have appeared later than those originally from the insect native range. Accordingly, and although we must be cautious before making any statement with the existing data, our results show that the basal position of the phylogenetic tree (closest to the outgroup) is occupied in all cases by haplotypes collected at least once in Europe (Figure 2).

In our study, the COI (full gene) genetic diversity was higher than in some Asian areas in which the species has been introduced (Li et al., 2015). The low error rates of Sanger Sequencing, only one error every 10,000–100,000 nucleotides (Kircher & Kelso, 2010), reduce any doubt in the veracity of the results based on the sequencing methodology. The repeated introductions from the Mediterranean European coast may have favoured the higher genetic diversity in Algeria compared to China (Dlugosch & Parker, 2008). Alien species populations usually show a low genetic diversity in the invaded areas due to founder effects (Bonal et al., 2018; Dlugosch & Parker, 2008, review). Quick population expansion from an initial low number of founder individuals is also typical in invasions, and usually leaves a genetic signature in terms of significant and negative Tajima's D and/or Fu's F (Ferrando et al., 2015). We did not find any significant value of these two parameters in our study, which supports the idea of recurrent introductions from Europe.

Contrary to other studies in invaded areas (e.g., Cichón et al., 2021; Li et al., 2015), we found no genetic differentiation among the three sampling localities in Algeria. This is not surprising since, compared with those studies, the distance between populations was not as large (50 km maximum) and the markers used (mitochondrial DNA) have a lower resolution to detect differences compared with DNA microsatellites (SSR) (Cichón et al., 2021). Functional genes, such as mitochondrial DNA, have a mutation rate too low to detect recent gene flow constraints. Only if different COI haplotypes had been introduced at different localities and we had seen that such segregation exists today, we could presume lack of gene flow. However, that segregation could be undetected but gene flow constraints still occur. For example, nuclear markers (e.g., SSR) could still tell us whether gene flow constraint could be occurring/have occurred if there was a significant allele segregation among localities. Thus, further studies with markers with higher mutation rates (e.g., SSR and Single Nucleotide Polymorphisms (SNPs)) will be able to assess the real extent of inter-population gene-flow in Algeria.

In conclusion, the present study provides, for the first time, sequences of DNA barcodes for African populations of *C. pomonella* and shows that the origin of the invasive populations may be Europe. Most mitochondrial COI haplotypes were shared with this continent, but the presence in Algeria of two exclusive haplotypes (singletons), closely linked to the most prevalent one, suggest that these might have originated locally (probably favoured by high pest mutation rates). Further studies should increase the geographical coverage of DNA barcoding to other North African countries where the species occurs (MeniMahzoum et al., 2018). Moreover, it could be promising to extend research to functional nuclear genes involved in adaptive responses to changing environmental conditions (Wan et al., 2019). The time since introduction, and our finding of potential local mitochondrial mutants, increase the chance of finding nuclear gene variants which might favour adaptation to the local conditions of Northern Africa.

AUTHOR CONTRIBUTIONS

Tayeb Mahi: Conceptualization; investigation; methodology; resources; writing – original draft. **Abdelkader Harizia:** Funding acquisition; investigation; methodology; project administration; resources. **Tara Canelo:** Investigation; methodology. **Abdelkader Benguerai:** Investigation; project administration; resources. **Raul Bonal:** Conceptualization; formal analysis; funding acquisition; investigation; methodology; resources; software; writing – original draft.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The new DNA barcodes sequenced in this study have been uploaded to a public repository (GenBank https://www.ncbi.nlm.nih.gov/genbank/) in which they freely available (accession numbers shown in the Results section).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Accession numbers of the sequences downloaded from Barcode of Life.

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