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Patterns of *Vespa velutina* invasion in western Iberia and Italy as revealed by mitochondrial and microsatellite markers

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"All animals are equal, but some animals are more equal than others"

George Orwell

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Abstract

The Yellow-legged or Asian hornet, *Vespa velutina nigrithorax*, is naturally distributed in Southeast Asia, and it is the first successful exotic Vespidae predator to be accidently introduced in Europe. In 2004, the first individual was reported in France, probably from China and, in the last decade, it spread rapidly through the French territory and to other European countries. In the Iberian Peninsula it was reported for the first time in Spain, in 2010, and in Portugal, in 2011. In 2012, two individuals were reported in the Italian region of Liguria. Using a population genetics framework, the goal of this study was to test the genetic patterns of colonization of this invasive honey bee predator in the Atlantic side of Iberia and in Italy. A total of 246 individuals, each representing a single colony, were collected across the invaded areas in Portugal (190), Spain (45), and Italy (11). Additionally, a dataset containing samples from France, Vietnam, South Korea, Indonesia and two provinces of China provided by Arca et al. (2015) was used as a reference for testing hypothesis about the origin of the invasion and the expansion patterns. The genetic variability was assessed using 16 microsatellite loci and the mitochondrial DNA (mtDNA) cytochrome C oxidase I (COI). The mtDNA analysis revealed the presence of a single haplotype in Iberia and Italy, which has been also reported for France and the UK. Microsatellite analysis showed a low genetic diversity in these populations, as it is expected from an expansion of an invasive species. Population structure analysis showed that the European populations separate in two clusters: (i) one that contains the populations from France and Italy, and (ii) one with the Portuguese individuals. The Spanish population has individuals in both clusters, while the Portuguese population showed some migrants that cluster with the French individuals. Thus, it was demonstrated that the expansion of the Asian hornet is different in the three countries. Spain and Italy showed a natural spread of the hornet, whereas the Portuguese population showed a human-mediated diffusion together with a natural spread of the Asian hornet.

Keywords: Asian hornet; yellow-legged hornet; invasive species; population genetics; molecular markers

Resumo

A vespa asiática, *Vespa velutina nigrithorax*, encontr-se naturalmente distribuída no sudeste da Ásia. Em 2004, tornou-se no primeiro predador exótico da família Vespidae a colonizar a Europa, ao ser introduzido acidentalmente em França e, na última década, propagou-se rapidamente pelo território francês e, bem como por outros países europeus. Na Península Ibérica, foi vista pela primeira vez em Espanha, em 2010, e em Portugal, em 2011. No ano seguinte, 2012, dois indivíduos foram encontrados na região italiana de Liguria. Tendo como base a genética populacional, o objetivo deste estudo foi testar os padrões genéticos de colonização desta espécie invasiva, que tem como principal presa as abelhas melíferas, no litoral Atlântico da Península Ibéria e em Itália. Um total de 246 indivíduos, cada um representando uma única colónia, foram recolhidos nas áreas invadidas de Portugal (190), Espanha (45) e Itália (11). Adicionalmente, um conjunto de dados contendo amostras de França, Vietname, Coreia do Sul, Indonésia e de duas províncias Chinesas, fornecidas por Arca et al. (2015), foi utilizado como referência para testar várias hipóteses sobre a origem e expansão desta espécie invasora. A variabilidade genética foi avaliada usando 16 microssatélites e o citocromo C oxidase I (COI) do DNA mitocondrial (mtDNA). A análise do mtDNA revelou a presença de um único haplótipo tanto na Península Ibérica como em Itália, o mesmo relatado para a França e o Reino Unido. Quanto aos microssatélites, estes mostram uma baixa diversidade genética nestas populações, como é de esperar de uma expansão de espécies invasoras. A análise da estrutura populacional mostrou que as populações europeias se separam em dois grupos: (i) um que contém as populações da França e Itália e, (ii) outro com os indivíduos portugueses. Os indivíduos da população espanhola encontram-se divididos pelos dois grupos, enquanto que a população portuguesa contém alguns migrantes que se estabelecem no grupo das francesas. Assim, foi demonstrado que a expansão da vespa asiática difere nos três países. Espanha e Itália mostraram uma propagação natural da vespa, enquanto que a população portuguesa mostrou uma difusão mediada pelo Homem juntamente com uma expansão natural da espécie invasora.

Palavras chave: Vespa asiática; espécies invasoras; genética da população; marcadores moleculares

I. Introduction

1. Framework

The Asian hornet, *Vespa velutina nigrithorax*, is a subspecies of *Vespa velutina* with the native range in Southern Asia, where it inhabits temperate zones (Leza et al. 2018). It produces annual colonies that start growing in spring when a hibernated queen begins to build a nest that will grow until autumn, reaching more than 10 000 workers and producing, at the reproductive season, around 900 males and 350 gynes (Kishi and Goka 2017). *V. v. nigrithorax* became an invasive species when it was introduced in France, at the beginning of the 2000's, probably through an imported pottery shipment from China (Villemant et al. 2006). Since then, it has rapidly spread throughout the European continent. In the Iberian Peninsula, the first individual was observed in 2010, first in Navarre and then in the Basque Country (Spain), through a natural expansion from France (Castro and Pagola-Carte 2010; López et al. 2011). One year later, in 2011, it was recorded the first individual in Minho (Portugal) where, according to anecdotal reports, its entrance was made through terrestrial transportation of wood (Grosso-Silva and Maia 2012). In 2012, *V. v. nigrithorax* was reported for the first time in Galicia (Spain) and in Liguria (Italy) (Demichelis et al. 2014). This hornet became well established in Europe and, due to its predatory characteristics, it has an important impact on the local biodiversity. As a specialized honey bee predator, it represents a serious threat to beekeeping because the peak of its activity occurs at the same time that honey bees are preparing to overwinter. By interfering with the production of the overwintering workers, the predation of the Asian hornet in autumn leaves the colonies weaker to survive the winter, thereby having an impact on the production of honey yield in the next year. In this context, measures for controlling *V. v. nigrithorax* are needed, and the efficacy of these measures can be improved by uncovering the genetic diversity patterns in the invaded area and determining the geographical origin of the invaders (Estoup and Guillemaud 2010). This is achieved by helping designing measures to control the entrance at the ports and by guiding the search of parasitoids that are natural enemies of the invasive species in the native environment. By providing important information about allelic variation at a given locus, the use of neutral molecular markers, such as microsatellites, which are highly variable and polymorphic, and mitochondrial DNA sequence variation, are suitable for uncovering the genetic diversity patterns of the Asian hornet.

2. Objectives

Using microsatellites and mitochondrial DNA (mtDNA) sequence variation as markers, the populations of *V. v. nigrithorax* in the invaded range of Portugal, North-western Spain and Italy will be surveyed. Microsatellites and mtDNA sequence data from a reference dataset developed for the introduced range in France and South Korea and for the native range in the Chinese provinces of Yunnan and Zhejiang/Jiangsu, Indonesia and Vietnam (Arca et al. 2015) will be used for comparisons. The objective of this study is to analyse the population structure and the genetic patterns of the invasion of *V. v. nigrithorax* in the three countries. In accomplishing this objective, we will address the following questions:

i. Is the origin of the Portuguese invasion from Asia or France?
ii. Does the genetic composition of the populations from Portugal and North-western Spain differ?
iii. Are there any differences between the populations from North and South of Portugal?
iv. How many genetic clusters do we find in Iberia and Italy?
v. How many haplotypes are present in Iberia and Italy?
vi. Is there evidence of new bottlenecks in Iberia and Italy?

II. Literature Review

1. Biological Invasions

Biological invasions have been increasing dramatically around the world in the last decades due to global change, worldwide trade, and human mobility. In fact, invasive alien species (IAS) are widely recognized as a significant component of human-caused global environmental change and are a major threat to biodiversity (Frankham et al. 2010; Villemant et al. 2011), having also a severe impact on agriculture and natural resources (Mack et al. 2000; Leza et al. 2018). IAS are a small number of founders that can arrive in a new habitat and have the ability to spread and increase a sufficient population density, even though they suffer a loss of their genetic diversity, and cause economic (estimated in hundreds of billions of US dollars each year), social and/or environmental impacts (Frankham et al. 2010; Beggs et al. 2011; Cini et al. 2018). Social insects, in particular some social Hymenoptera, are amongst the most invasive species. This is due to their easy transportation, with low probability of detection in human trades, combined with a number of biological and life history traits, such as: the ability of one fertilized queen (usually carrying the semen of several males) to establish a population, the exceptional dispersal abilities, high reproductive rates, broad diets and habitat ranges, effective predator defences, superior competitive abilities, their social organisation, long flight season, large foraging range, ability of buffering against environmental changes. Altogether, these features make social insects better fit to successfully establish and spread through new territories (Beggs et al. 2011; Goldarazena et al. 2015).

Focusing on social Hymenoptera, the polyandrous nature of many species is a particularly interesting trait that gives them an advantage to disperse into new environments. Polyandry is a type of mating system of some species, where a female mates with several males. Within the Hymenoptera order, there is the Vespidae family. This family comprises over 5 000 species, including almost all species of eusocial wasps, as well as, some solitary species (Beggs et al. 2011). It is divided into six subfamilies: Euparagiinae (10 species) and Masarinae (344 species), both with a solitary behaviour, Eumeninae (3 579 species), which comprises species with both solitary and eusocial behaviours, Stenogastrinae (58 species), which are facultative eusocial, and finally Polistinae (958 species) and Vespinae (69 species), which have an eusocial behaviour

(Beggs et al. 2011). In the latter subfamily, we can find the genus *Vespa*. There are 22 known species in this genus, of which 20 are naturally restricted to Asia and Oceania while the other two expanded westwards. *Vespa crabro* Linnaeus (1758), the European hornet, colonised naturally the whole Eurasia and *Vespa orientalis* Linnaeus (1771) reached the Mediterranean Basin (Figure 1) (Beggs et al. 2011). Out of the 34 vespid species identified by Beggs et al. (2011) in introduced range around the world, 23 (68%) are eusocial and, all invasive alien vespid species are eusocial. This indicates that sociality favours the invasiveness, giving to the species a plasticity of responses that allow them to survive and evolve in a new environment.

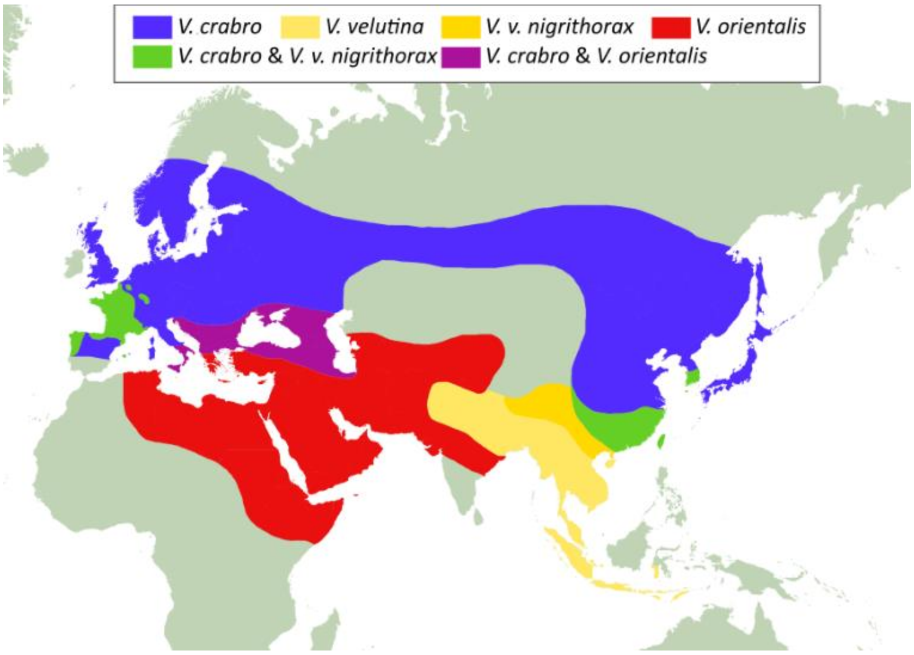

Figure 1: Distribution map of the two Vespidae species that naturally reached Europe and the Mediterranean basin (*V. crabro* and *V. orientalis*) and of the invasive species (*V. v. nigrithorax*). Source: https://www.vespavelutina.eu/en-us/

2. *Vespa velutina* ecology and life history

Vespa velutina Lepeletier (1836), yellow-legged or Asian hornet, is widespread through the whole Asian continent and there are 14 described subspecies (Kishi and Goka 2017). The subspecies *Vespa velutina nigrithorax* du Buysson (1836) is present in Southeast Asia, inhabiting temperate zones associated with forests, at altitudes of 200–800 m, even though it is possible to find them at lower or higher altitudes (López et al. 2011; Goldarazena et al. 2015; Leza et al. 2018). *V. v. nigrithorax* is easily distinguished from other Vespidae species, such as the European hornet (*Vespa Crabro*). It has a size between 19-30 mm, and it is characterized by a dark brown, almost black thorax, the front part of the head is yellow-orange and antennae are black at the ends and brown on the bottom (Figure 2). The first three abdominal segments are dark brown with a yellow backside margin and the fourth segment is almost entirely yellow-brown. The legs are dark, except for the ends that are yellow, a characteristic that gave this species the name yellow-legged hornet. The males of *V. v. nigrithorax* can be differentiated from workers or queens for the absence of the sting and bigger antennas. On the other hand, workers and queens are very similar, being necessary to perform an internal analysis of the reproductive organs to allow their definitive distinction (http://www.vespavelutina.eu/en-us/). The queens can fly about 30 km in one day, which gives them the advantage of possessing a high dispersion capability (Beggs et al. 2011).

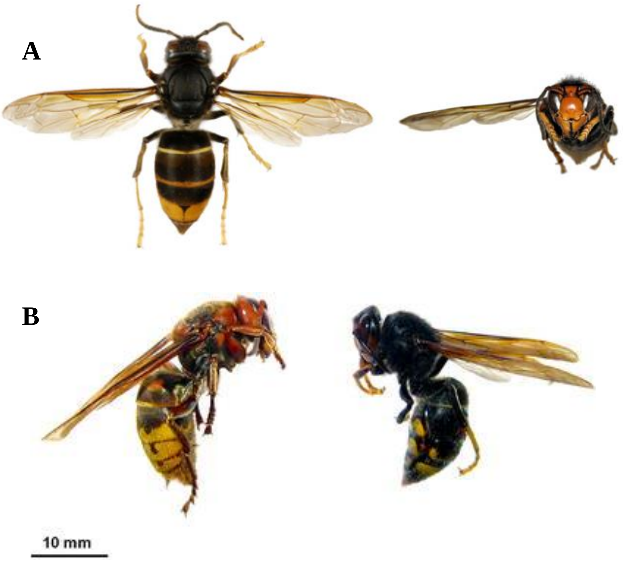

Figure 2: Dorsal and anterior view of V.velutina (A) and queens from V. crabro (left) and V. velutina (right) (B) Source: Monceau et al. (2014)

Based on microsatellite data, Arca et al. (2015) demonstrated that *V. v. nigrithorax* is a polyandrous species. This recent finding makes this hornet as the only species of the genus Vespa to present a moderate polyandry, whereas *V. crabro* and *Vespa mandarinia* Smith (1852) present a facultative polyandry, and *Vespa ducalis* Smith (1852) is monandrous. Therefore, a single female of *V. v. nigrithorax* has the advantage of carrying more genetic diversity and, thus, is better equipped to survive and establish in new environments.

V. v. nigrithorax produces annual colonies, which begin when a hibernated founder queen starts to build a primary nest in early spring (~March), using fibrous origin substances and saliva. Primary nests are normally found in cavities underground or in bushes near the ground. During this phase, called “the queen colony phase”, the queen is the only responsible for building the nest, laying the eggs, feeding the brood and producing, approximately, a hundred workers (Monceau et al. 2014; Turchi and Derijard 2018). This is a delicate phase since the queen is alone and vulnerable, thus it has a high probability of failing establishing the colony. After the first workers start emerging, at the beginning of the summer (~June), the primary nest is abandoned and the colony moves to a higher site, usually more than 10 m above the ground, where it builds a secondary nest (Monceau et al. 2014). Secondary nests are built preferentially in the tree canopy, but they can also be found in shrubs, balconies or roofs of houses. The size of the secondary nest can reach one meter in length and a colony can produce hundreds to thousands of workers. Secondary nests can be discovered either in natural areas, as well as rural or urban areas. A study of 550 nests, in France, indicated that 90% had been built in the top of trees, with the remaining 10% in buildings. Preferred tree species for building the nest are oaks, poplars, acacias and conifers. Reports of nests show that they are higher in cities and urban areas, due to increased awareness of the people, while the high amount of vegetation in natural areas makes spotting nests difficult, since most of them are hidden and are only seen in when leaves fall (Mollet and de la Torre 2007; http://www.vespavelutina.eu/en-us/; Goldarazena et al. 2015). In autumn (September – November), the nest reaches its larger size. At this time of the year, the reproductive season starts and, most activities are related to mating and dispersal. Approximately, 900 males and 350 gynes (new generation of queens) emerge from the nest, i.e., an average of three times more males than gynes. Before winter, the colony dies (males and workers) and the mated gynes take shelter in small cavities, where they will survive the winter. This is also a fragile phase for the gynes since overwintering mortality can be high and only some of the gynes will emerge in the next spring (Figure 3) (Mollet and de la Torre 2007; López et al. 2011; Monceau et al. 2014; http://www.vespavelutina.eu/en-us/; Kishi and Goka 2017).

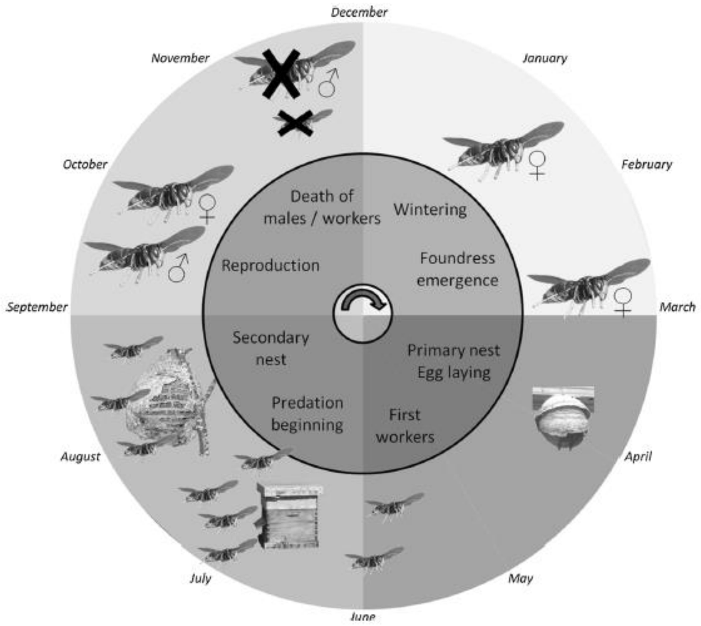

Figure 3: Life cycle of *V. velutina*. The crosses on males and worker in December represent their dead during this time, surviving only the gynes. Source: Monceau et al. (2014)

In its native environment, *V. v. nigrithorax* is a generalist predator, consuming a large variety of insects (e.g. honey bees, Vespula spp., Diptera and spiders) and scavenging a variety of protein sources (e.g. like fish or shrimp and fruits) (Beggs et al. 2011). The larvae are fed with insects while the adults need carbohydrates as an energy resource consuming, therefore flower nectar, tree sap and juice of ripening fruit (Kishi and Goka 2017). When *V. v. nigrithorax* finds an apiary, it tends to specialise in honey bees as its prey. The hornets occupy a position above a beehive, hovering outside of the colonies, in a stationary flight, and catch foraging honey bees when they return to the hive, a behaviour known as bee-hawking (Beggs et al. 2011; Keeling et al. 2017). Each hornet vigorously defends its hunting territory, chasing off any rivals that might approach. However, after catching a bee, the hornet flies off and another one replaces it. The circadian activities of the predator and honey bee prey are similar, having the most intense activity occurring during the morning and afternoon (Tan et al. 2007). After catching the honey bee, the hornet flies to the nearest branch where it cuts its prey, keeping only the thorax which contains the nutrition flight muscles that will be fed to the larvae (Beggs et al. 2011).

V. velutina is able to destroy up to 30% of a colony of the Asian honey bee (*Apis cerana* Fabricius 1793) (Mollet and de la Torre 2007; Beggs et al. 2011). But as a co-evolved species of the Asian hornet, *A. cerana* developed efficient defence strategies, such as: the heat-balling, the bee-carpet and the abdomen shaking movement/shimmering. In the heat-balling strategy, the honey bees pack themselves into a compact ball around the hornet, and by contracting their thoracic muscles, they will increase the core temperature of the ball around 45ºC and, consequently, kill the hornet. The bee-carpet strategy consists in the formation of a large aggregation of honey bees at the hive entrance. The bees will try to catch the hornet with their front legs and mandibles and, if they are successful, they will form a heat-balling around the hornet. In the abdomen shaking movement/shimmering strategy, Asian honey bees at the entrance of the hive simultaneously vibrate their abdomens for a few seconds, after spotting the predator, resulting in the emission of a loud hissing noise that serves as an alarming signal (Mollet and de la Torre 2007; Baracchi et al. 2010; Nouvian et al. 2016; Monceau et al. 2017; Pusceddu et al. 2017). On the other hand, the western honey bee *A. mellifera*, which was introduced in the early 20th century in Asia, only exhibits the bee-carpet and the heat-balling strategies, but the bees are less effective defending themselves against the hornet as they did not co-evolve together. In Europe, where *A. mellifera* is native, the same inefficient defence of the colonies is observed (Mollet and de la Torre 2007; Beggs et al. 2011; Keeling et al. 2017; Monceau et al. 2017), which can also be explained by beekeepers-mediated selection favouring the calmness of honey bees. Selection for gentleness have made the bees less aggressive leading to an inability to attack their predators in front of the hive entrance and to rapidly recruit a sufficiently large number of honey bees for a heat-balling behaviour (Arca et al. 2014). Therefore, *A. mellifera* is not prepared to deal with this predator in both native and introduced ranges.

3. Invasion dynamics of *Vespa velutina*

V. v. nigrithorax has recently invaded Europe becoming the first successful exotic Vespidae predator to be accidentally introduced in this continent (Villemant et al. 2011; Monceau et al. 2014). The very first individual was reported in France in 2004, in Lot-et-Garonne (Villemant et al. 2011). Since then, *V. v. nigrithorax* has rapidly spread through the southwest of France and it is now well established in the country. According to

Villemant et al. (2006), the accidental introduction is linked to an imported pottery shipment from China. The hypothesis of an origin in China is supported by genetic data. Furthermore, the strength of the genetic bottleneck, as well as, the presence of a single mitochondrial haplotype in Europe also suggests the introduction of few or a single mated foundress (Arca et al. 2015).

In 2010, the hornet arrived in Spain. The first individual (a female) was reported in Navarre and, later on that year, in the Basque Country (Castro and Pagola-Carte 2010; López et al. 2011). Introduction and expansion in Northeast Spain appear to have been by natural dispersal. Although the Pyrenees have acted as a natural barrier for the dispersal from France, an invasion via the northern valleys of the border, which have a lower altitude (around 800 m), facilitated an entrance in Spain (Castro and Pagola-Carte 2010; Goldarazena et al. 2015). In 2011, a single male of *V. v. nigrithorax* was seen in Belgium, close to the French border. However, since this was a single observation, *V. v. nigrithorax* does not seem to have established itself in the country (Bruneau 2011; Scalera et al. 2012) until 2016 when a nest was found in Guignies, near the French border (EPPO Reporting Service 2017/145). In 2011, the first individual was spotted in Minho, Portugal (Grosso-Silva and Maia 2012). Anecdotal reports point to an entrance through terrestrial transportation of wood. In the following year, 2012, the first individual was reported in Galicia, Spain and two individuals were observed in Liguria, Italy (Demichelis et al. 2014). According to Bertolino et al. (2016) the entrance of the Asian hornet in Italy may have occurred through a narrow corridor along the North-western coast, since the Alps act as a barrier at the border between France and Italy. *V. v. nigrithorax* reached Germany in 2014 (Witt 2015), the UK in 2016 and Switzerland in 2017 (Budge et al. 2017; Keeling et al. 2017). A map with the current distribution of the Asian hornet in Europe is shown in Figure 4.

V. v. nigrithorax also invaded neighbouring countries in Asia: in the 2000s arrived in South Korea (Kim et al. 2006) and in 2012 in Japan (Sakai and Takahashi 2014). In South Korea, the spread of the hornet was slow, which might be explained by the presence of a richer local hornet community, with six other species, that could compete with the new comer. This contrasts with the invaded area in Europe, where so far, the Asian hornet only has the native *Vespa crabro* to compete with (Bertolino et al. 2016). According to analyses of nuclear markers, the *V. v. nigrithorax* that invaded South Korea are more

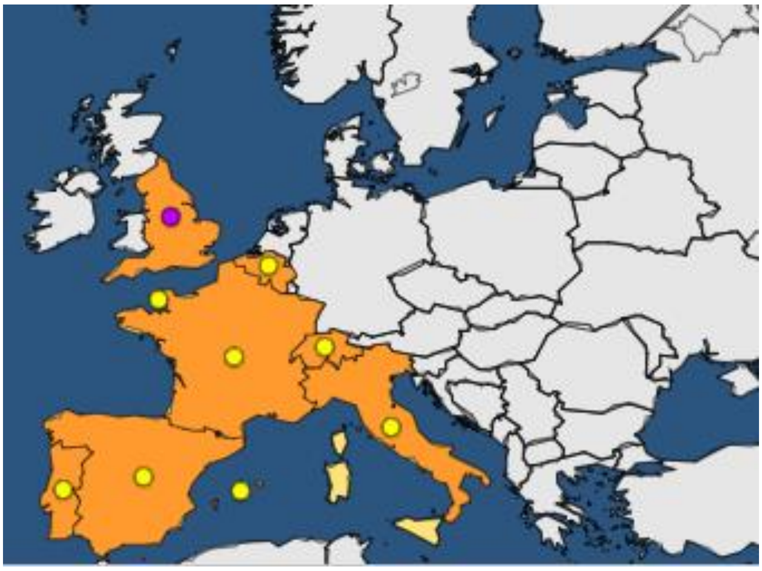

Figure 4: Current distribution of the Asian hornet in Europe. Source: EPPO

closely related to the one that also invaded France than to other Asian populations (Arca et al. 2015), suggesting China as the origin of the South Korean invasion.

The spread of *V. v. nigrithorax* throughout Europe was characterized by a combination of a natural dispersal with a diffusion-like process and sudden jumps due to the passive transport of gynes mediated by humans (Monceau et al. 2014). This is linked to the use of a variety of transportation types (terrestrial, maritime, aerial), which makes the spread difficult to predict. On the other hand, the natural dispersal determines the progressive spread of colonies and can be evaluated and modelled from data on the diffusion over successive years (Bertolino et al. 2016).

Studies concerning the modelling of the potential invasion extent of *V. v. nigrithorax* based on native and invasion records showed that almost all European countries could be colonised by the Asian hornet, but with a reduced risk at the drier southern regions (Beggs et al. 2011). However, inferring from the current invaded area since introduction in France, it has been hypothesized that the hornet will not face any barrier to the neighbour countries, and will spread preferentially along the Atlantic and the Mediterranean coasts and the Southern coasts of Black and Caspian Seas (Rome et al. 2011; Villemant et al. 2011; Barbet-Massin et al. 2013). Barbet-Massin et al. (2013) estimated the invasion risk under climate change scenarios and predicted an increase in the climatic suitability across central and eastern Europe and, therefore an increased risk in these areas.

4. Impact of *Vespa velutina*

a. Predation

The Asian hornet is considered an IAS for its impact on biodiversity, beekeeping and human health (Bertolino et al. 2016). Impacts linked to its invasion in Europe can range from: (i) economic, due to interference with pollination and honey production. For example, in France, bee pollination services are estimated in 1.5 billion €/year and the direct loss for beekeepers is calculated to be over a 100 million €/year; (ii) public health, due to the possibility of existing an allergic reaction to its sting that can cause death. However, the number of cases does not seem to have increased with the *V. v. nigrithorax* expansion; to (iii) ecological, due to the predation not only on honey bees, but also on a vast array of native insect species, some of which provide valuable ecosystem services, such as pollination (Monceau et al. 2014; Turchi and Derijard 2018).

Although *V. v. nigrithorax* is known as a threat to honey bees by direct predation, the impact on colony foraging and defence activities has not yet been investigated in Europe (Monceau et al. 2017). However, field observations point to an intensive predation on foragers during late summer and autumn, coinciding with the time that colonies are producing their overwintering population. Thus, the predation by the Asian hornet represents a further threat to honey bee populations, which are already suffering a significant decline throughout Europe due to several factors.

b. Competition

In addition to the direct impact on local biodiversity due to predation, IAS have a large effect, more difficult to assess, related to competition. This can be mediated either by second-order ecological interactions, such as, indirect dispersal and transmission of pathogens or parasites, or by competition for space and other resources between the IAS and the native species. The latter often occurs when the introduced IAS occupies an ecological niche very similar to the one filled by a native species. Concerning the second-order ecological interactions, IAS might either benefit from the absence of specialised pathogens or suffer from the presence of pathogens with which they did not co-evolve with, a theory known as the “enemy release hypothesis”. Another hypothesis, called the “evolution of increased competitive ability” (EICA), proposes that an invasive species is subjected to less predation and parasitization than sympatric native species, which allows the IAS to allocate resources from defence and immunity to growth and fecundity, thereby achieving higher fitness (Cini et al. 2018).

The European hornet, *V. crabro* is considered an endangered species in several European countries and it faces a new important competitor in the invasive hornet, *V. v. nigrithorax*, both for space and resources therefore, increasing its threat (Villemant et al. 2011). These two Vespidae species have a similar life cycle, being the two main differences the length of the annual life cycle, which is longer in the Asian hornet (from February/March to November), and the size of the colony, with the Asian hornet building larger nests with a higher number of individuals.

The competition between the two species may occur in two phases: colony foundation and colony growth. Concerning colony foundation, competition is due to the higher explorative tendencies of *V. v. nigrithorax* queens. In the case of colony growth, *V. v. nigrithorax* may outcompete *V. crabro* for resource exploitation during summer and autumn because of its foraging strategy, with a high number of workers foraging and defending food sources. Moreover, the different predation strategies and the fact that *A. mellifera* is able to defend itself from *V. crabro* attacks and that *V. v. nigrothorax* drastically outnumbers *V. crabro* in both colony density and colony size, it is expected that the European hornet may be replaced by the Asian hornet. Furthermore, Cini et al. 2018 showed that *V. crabro* workers have higher feeding rates when it comes to carbohydrate sources, whereas *V. v. nigrithorax* workers spend more time in protein sources. This could be explained by differences in physiology and morphology. The differences in feeding rates is in accordance with the hypothesis of the invasive species outperforming the native one in foraging, since protein supply is collected to feed the developing brood, while carbohydrates are used to the workers own energy. The same study also showed that workers of the European hornet were significantly more immunocompetent than the Asian hornet workers in respect to the ability to remove bacteria from their haemolymph which, according to the authors, might be linked to a higher degree of inbreeding in the invasive species. The difference in food resources preference may also be explained by the fact that *V. v. nigrithorax* workers do not have natural enemies in the invasive areas, so they can afford to invest their resources in other activities rather than immunity. Therefore, they need a lower consumption of high-energy carbohydrates when compared with *V. crabro* workers.

5. Importance of the history of the invasion routes

An introduced organism will encounter different ecological conditions in its new environment. Thus, the success of the invasion may be determined by natural selection and adaptation, which in turn will depend on several factors and one of them is the genetic variability. By knowing the genetic variability of an invasive population, we can infer the historical and demographical features of the introduction (Estoup and Guillemaud 2010). Understanding the history of the invasion and, therefore reconstructing the invasion routes, has both practical and academic implications. From a practical point of view, it facilitates the design of strategies to prevent future invasions, and to control present invading populations, since the efficacy of these measures depends on the genetic diversity and geographical origin of the introduced species (Estoup and Guillemaud 2010). From an academic point of view, it is crucial for defining and testing different hypotheses on environmental and evolutionary factors underlying biological invasions. For example, founder effects among source populations can have important consequences for evolution in the introduced area, as well as, introducing a bias in the inference of which forces shaped the invaded population structure during the establishment phase. This will result in changes at a phenotypic level of the introduced population (Keller and Taylor 2008).

6. Molecular markers

Conservation genetics is an applied science that uses molecular tools to help solve problems in species conservation, management, and biological invasions (Hedrick and Miller 1992). It includes the fields of population genetics, molecular ecology, phylogenetics, phylogeography, and taxonomy (Larson and Lowry 2017). The field of population genetics has become important for investigating biological invasions by providing the theoretical framework for deciphering migration pathways and demographic mechanisms underlying the colonization process (Choi et al. 2013). For these analyses, population genetics uses neutral molecular markers, which are highly variable and polymorphic, thus providing information about allelic variation at a given locus.

Advances in molecular biology led to the introduction of many types of molecular markers (Schlötterer 2004), such as, microsatellites, Single Nucleotide Polymorphisms

(SNPs), which are biparental inherited, or mitochondrial DNA (mtDNA), which is of maternal heritage. Bellow, two of the most widely used molecular markers will be described: microsatellites and mtDNA.

a. Mitochondrial DNA

In the early 1980’s, John Avise authored several seminal papers using mtDNA as a marker in his studies of phylogeography, popularizing this term (DeSalle et al. 2017). Since then, this small (< 20 000 bp in animals), double-stranded, circular and haploid molecule, which is present in all eukaryotic cells, became widely used as a molecular marker in evolutionary studies due to its unique characteristics. In animals, mtDNA is maternally inherited and non-recombinant, codifying proteins used for gene transcription and cellular respiration (Larson and Lowry 2017). Although mtDNA evolves at a rate four times faster than nuclear DNA, the rate of evolution is heterogeneous across the molecule, varying from moderately slow in the cytochrome c oxidase I gene (COI), for example, to moderately fast in the A+T-rich region segment of the mitochondrial control region. This is one of the reasons for the popularity of mtDNA in population genetics studies (Larson and Lowry 2017; DeSalle et al. 2017). Another reason is because, due to several copies in the cell, mtDNA is easily isolated and restriction enzymes as well as sequencing can be used to detect nucleotide differences between individuals. The characteristics of this marker are summarized in table 1 and its advantages and disadvantages in table 2.

In *V. v. nigrithorax*, the mitochondrial molecule is around 16 000 bp, which is largest than other Vespidae species. It includes the typical set of genes: two rRNAs, 22 tRNAs and, 13 protein coding genes. The average AT content of *V. v. nigrithorax* mitogenome is 81.9% (Kim et al. 2017; Takahashi et al. 2017).

The COI gene has a slower mutation rate when compared to other protein coding mitochondrial genes and has modest within-species diversity. This feature makes COI a useful marker for identifying divergence between species, with the advantage that conserved regions within the gene allow designing of robust universal primers (Taylor and Harris 2012; Patwardhan et al. 2014). Due to these characteristics, a sequence of approximately 600 bp of the COI gene was proposed as the best candidate for DNA barcoding (Hebert et al. 2003), and robust universal primers for PCR amplification were made available for surveying variation in animals. DNA barcoding has shown a higher taxonomic resolution than morphology, providing a better way of not only identifying species but also defining species boundaries (Hebert et al. 2003; Taylor and Harris 2012).

Since Hymenoptera is a taxonomically challenging group, COI-barcode has been widely used to identify cryptic species (Williams et al. 2012; Wilson and Schiff 2010; Mitrović and Tomanović 2018), a faster identification of species (Vargas et al. 2014; Turčinavičienė et al. 2016; Mitrović and Tomanović 2018), discover new species (Sheffield et al. 2009) and, create taxon-specific and region-based species banks (Sheffield et al. 2009).

Arca et al. (2015) used COI-barcode to study the origin of *V. v. nigrithorax* in the invaded countries of France and South Korea. Eleven haplotypes were detected from four native regions (Zhejiang/Jiangsu and Yunnan (China), Vietnam and Indonesia). However, only two of them were found in the invaded areas: haplotype F in France and haplotype K in South Korea. COI-barcode was also used in the individuals of *V. v. nigrithorax* found in the UK and the same haplotype F from France was described (Budge et al. 2017). Takeuchi et al. (2017) analysed the COI segment of the individuals found in the islands of Tsushima and Kyushu, Japan, and only found the haplotype K, the same one found in South Korea.

b. Microsatellites

Microsatellites, also known as Simple Sequence Repeats (SSRs) or Short Tandem Repeats (STRs), are tandem repeated motifs of 2-6 bp found in prokaryotic and eukaryotic genomes. Microsatellites are abundant in the nuclear genome of animals (e.g. 3% of the human genome), have a high degree of length polymorphism, and a higher mutation rate (10^{-2} to 10^{-6} mutations per locus per generation) than other nuclear and mtDNA markers (Wright and Bentzen 1994; Ellegren 2004). They are present in coding and non-coding regions and, are assumed to be functionally neutral (Moniruzzaman et al. 2015; Larson and Lowry 2017).

Depending on the architecture of the repeated sequence, microsatellite can be classified in four types: (i) perfect, when the repeat sequence is not interrupted by any base that does not belong to the motif; (ii) imperfect, if there is a pair of bases between the repeated motifs that does not match with the motif sequence; (iii) interrupted, when there is a small sequence within the repetitive sequence that does not match the motif sequence; and (iv) composite, if the sequence contains two adjacent distinctive sequence repeats (Bhargava and Fuentes 2010).

A fast and easy detection of microsatellites is possible by PCR, even for highly degraded DNA samples, due to their small size and high number of loci available in all species (Vignal et al. 2002).

Analysis of a given microsatellite is based on its length (allele), i.e., the higher the number of repeats, the higher the length of the sequence. By using capillary electrophoresis, it is possible to analyse the sequence length and to distinguish a heterozygous from a homozygous individual, depending on whether it carries two alleles with a different number of repeats or two alleles with the same number of repeats, respectively. In other words, microsatellites are co-dominant markers and thus suitable for population genetics analyses involving testing of Hardy-Weinberg Equilibrium. The characteristics of this marker are summarized in table 1 and its advantages and disadvantages in table 2.

It is assumed that microsatellites' principal mechanism of mutation is a strand slippage during DNA replication, which results in the gain or loss of repeat unit(s) (Putman and Carbone 2014). It has been reported that mutation rate tends to increase exponentially with the length of the sequence, i.e., the higher number of repeats the higher the mutation rate (Bhargava and Fuentes 2010).

Inference in population genetics often requires modelling of the mutational process and, thus, some biologically realistic models were developed for microsatellites. Classical population genetics theory is mostly based on the infinite allele model (IAM). In this model, each mutation event results in a new and unique allele, and mutation at a given locus is assumed to occur only once. Single nucleotide substitutions detected by gene fragment sequencing, and more recently by array-based technologies can be analysed using the IAM model (Kimura and Crow 1964; Tajima 1996; Putman and Carbone 2014). However, due to the mutational mechanism, the IAM is not suited to microsatellites, being a poor descriptor of the process, as alleles do not arise independently of the previous ones, but they do have a mutation history (Putman and Carbone 2014). Another theoretical model normally used is the stepwise mutation model (SMM), where each mutational event results in the gain or loss of a single repeat unit (Ohta and Kimura 1973; Slatkin 1995). However, even though SMM fits the microsatellite mutational process better than the IAM, it still does not quiet describes it. Therefore, Di Rienzo et al. (1994) proposed a model that falls in-between the two models, which is called the two-phased model (TPM). By using TPM, which considers the variance of the microsatellite evolution among loci and the behaviour of a given locus, estimation of allele frequency distributions becomes more accurate (Ellegren 2004; Sainudiin et al. 2004; Putman and Carbone 2014).

During the 1990's, microsatellites gained popularity in population studies, due to their unique characteristics, and they have been used in genetic fingerprinting, parentage identification, genetic mapping, conservation and population genetics (Putman and Carbone 2014; Larson and Lowry 2017). While microsatellites have been recently superseded by SNPs in many applications, due to their high level of polymorphism they are more suitable for analyses of invading populations that have gone through a founder effect, such has occurred with the *V. v. nigrithorax* invasion in Europe.

After the establishment of *V. v. nigrithorax* in Europe, studies about the genetic variability of this species were necessary to describe its history of the invasion route. With that in mind, Arca et al. (2012), developed 15 microsatellites and tested them in individuals from France and China. The loci from France show a low number of alleles, as it is expected from an invasive population. To the 15 developed microsatellites, Arca et al. (2015) add seven microsatellites previously developed for other Vespidae (Hasegawa and Takahashi 2002; Daly et al. 2002). Using the 22 microsatellites, Arca et al. (2015) estimated the history route of the Asian hornet and, the occurrence of a founder effect. Budge et al. (2017) used 15 of the 22 microsatellites mentioned above to study the genetic diversity of the individuals found in the UK.

Table 1: Comparation of the features of the two molecular markers described

Marker	Features
mtDNA	Small genome size (normally < 20 000 bp) High number of DNA copies Maternally inherited Haploid inherited Non-recombinant
Microsatellites	High polymorphism Ubiquitous occurrence Randomly distribution in the genome

Table 2: Comparation of the advantages and disadvantages of the two molecular markers described

Marker	**Advantages**	**Disadvantages**
mtDNA	High mutation rate	Biased viewed of population history
	Easy isolation and manipulation	
Microsatellites	High rate of mutation	Species-specific
	Highly informative	Development of primers is expansive and time consuming
	Neutral	Cross-study comparisons require special preparation
	Easy to isolate	Requires calibration across labs
	Co-dominant marker	
	Can be used with degraded material	

III. Material and methods

1. Sampling and DNA extraction

A total of 246 adult female hornets were collected in Portugal (N = 190), Spain (N = 45), and Italy (N = 11) from founding nests, secondary nests, and mostly from traps placed in apiaries (Figure 5, Table S1). Most samples were collected between March and December of 2016 and 2017 (Table S1). Apiaries were over 3 km far apart to assure that sampled hornets originated from different nests. Samples were placed in absolute ethanol and stored at -20ºC until molecular analysis.

Total DNA was extracted from the thorax of the 246 individuals, each representing a single colony, using the Ron's Tissue DNA Mini Kit (®Bioron), according to the protocol provided by the manufacturer.

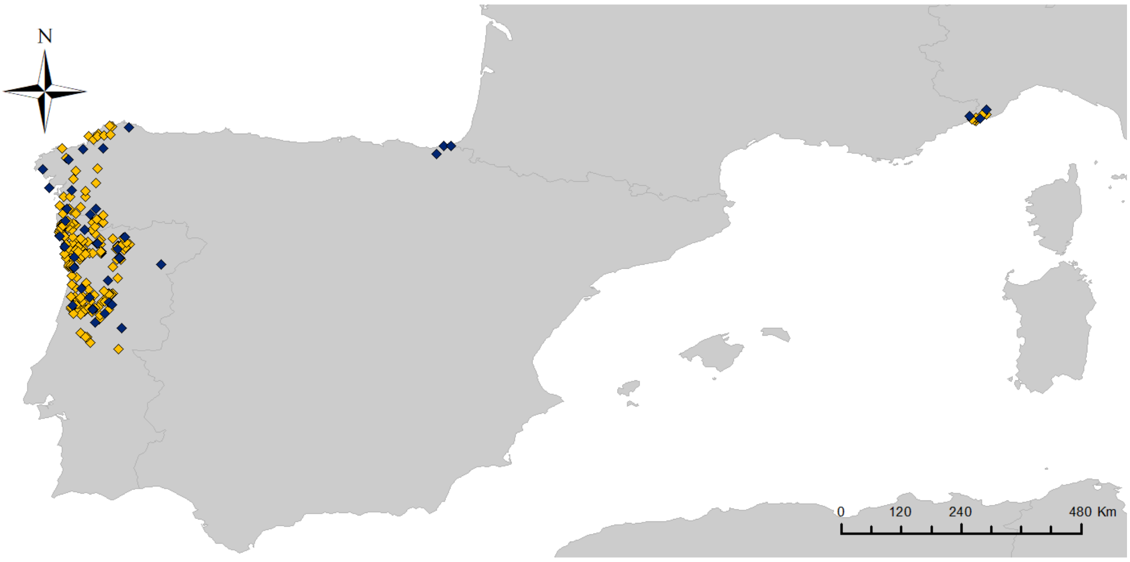

Figure 5: Map showing the spatial distribution of the sampled hornet colonies (N = 246) collected in Portugal (N = 190), Spain (N = 45), and Italy (N = 11), which were genotyped at 16 microsatellite loci. The blue dots represent the samples (N = 38) that were further examined using the 642 bp barcode Cytochrome C Oxidase Subunit I (COI) region of the mitochondrial DNA

2. Mitochondrial DNA analysis

A sub-sample of 38 individuals, which were selected to cover a wide geographical range (Figure 5, Table S1), was amplified at the mitochondrial DNA (mtDNA) 642 bp barcode region of the Cytochrome C Oxidase Subunit I (COI) gene, using the universal primers LCO-1490 and HCO-2198 (Folmer et al. 1994). Polymerase chain reaction (PCR) amplification was carried out to a final volume of 25 µL containing 0.2 mM of dNTPs (Promega), 2 mM of $MgCl_2$, 1X *Taq* DNA polymerase buffer, 0.2 µM of each primer, 0.625U of GoTaq®Flexi DNA polymerase (Promega) and 1 µL of DNA (concentration as extracted). PCR was performed in a T100 Thermal Cycler (BioRad) using an initial denaturation step of 94ºC for 5 min followed by 35 cycles of 30 sec at 94ºC, 45 sec at 50ºC and 1 min at 72ºC, and a final extension of 10 min at 72ºC. PCR products were sent to STABVIDA Inc. (Portugal) for direct Sanger sequencing in both directions. DNA sequences were checked manually for base calling and aligned using MEGA 7.0.26 (Kumar et al. 2016).

The 38 sequences were aligned with 23 sequences of *V. velutina* sequences from GenBank and a Maximum Parsimony (MP) phylogenetic tree was generated using MEGA 7.0.26. The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei and Kumar 2000) with search level zero in which the initial trees were obtained by the random addition of sequences (10 replicates). The resulting tree was rooted with *Vespa bicolor* (accession KT257112), *Vespa vivax* (accession KT257116) and *Vespa affinis* (accession KJ147242).

3. Microsatellite analysis

The 246 individuals were genotyped at 21 microsatellite loci using the primers developed by Hasegawa and Takahashi (2002), Daly et al. (2002) and Arca et al. (2012). The forward primers were labelled with 6-FAM, HEX, Atto 550 or Atto 565 fluorescent dyes (Table 3). The PCR amplifications were performed in three multiplexes using a 10 µL final volume containing 1 µL of DNA (10 ng/µL), 5 µL of Multiplex PCR kit (Qiagen), and 1 µL of a 2 µM primer mix. Forward and reverse primer sequences, repeat motif, and multiplex composition are shown in Table 3. PCRs were performed in a T100 Thermal Cycler (BioRad) using a temperature profile consisting of an initial denaturation step at 95ºC for 15 min followed by 40 cycles of 95ºC for 30 sec, 55ºC for 1 min and 72ºC for 1 min, and a final extension of 60ºC for 60 min. PCR products were run at STABVIDA Inc. (Portugal) on an ABI 3730xl DNA Analyzer using LIZ500 as the internal size standard. The lengths of the fragments were determined using GeneMapper 3.7 (Applied Biosystems).

Table 3: Description of the 21 microsatellite loci and composition of the three multiplexes

Multiplex A

Locus	Repeat motif	Size Range	Dye	Reference
R1-36	(CT)14	99-119	HEX	Arca et al. 2012
R1-169	(CT)27	148-165	HEX	Arca et al. 2012
R4-114	(TC)15	122-152	6-FAM	Arca et al. 2012
D3-15	(TC)15	157-180	Atto 565	Arca et al. 2012
LIST2015	(CT)3 … (CT)6(CT)2	167-198	Atto 550	Daly et al. 2002
LIST2020B	(CT)23(N)37(CA)11	183-217	6-FAM	Modified from Daly et al. 2002
R4-33	(GA)20	199-225	HEX	Arca et al. 2012
R1-77	(CT)15(CTT)5	241-255	Atto 565	Arca et al. 2012

Multiplex B

Locus	Repeat motif	Size Range	Dye	Reference
LIST2018B	(GT)19	113-161	Atto 550	Modified from Daly et al. 2002
LIST2004B	**(CTT)13**	**120-152**	**HEX**	**Modified from Daly et al. 2002**
R1-137	(GA)23	168-202	HEX	Arca et al. 2012
R3-115	**(CT)16**	**140-184**	**PET**	**Arca et al. 2012**
R1-158	**(CA)20(CG)5**	**153-174**	**FAM**	**Arca et al. 2012**
D2-185	(CT)18	208-228	6-FAM	Arca et al. 2012
VMA-8	(CT)9	230-271	Atto 565	Hasegawa and Takahashi 2002

Multiplex C

Locus	Repeat motif	Size Range	Dye	Reference
R1-80	(GT)19	100-174	6-FAM	Arca et al. 2012
R1-75	(AC)14	142-154	Atto 565	Arca et al. 2012
LIST2003	**(CT)10**	**153-193**	**HEX**	**Modified from Daly et al. 2002**
R4-100	(AC)19(CT)6	154-194	Atto 550	Arca et al. 2012
R4-26	**(CT)21**	**229-272**	**6-FAM**	**Arca et al. 2012**
VMA-6	(CT)16	238-242	HEX	Hasegawa and Takahashi 2002

Microsatellites in bold were removed after quality control

To have an estimate of the amplification error rate, 30 random samples were genotyped twice and analysed with the GIMLET 1.3.3 software (Valière 2002). To identify genotyping errors caused by large allelic dropout, stuttering and/or the presence of null alleles, all genotypes were tested with MICRO-CHECKER (Van Oosterhout et al. 2004). The genotype dataset was merged with an existing dataset generated by Arca et al. (2015) from samples collected in introduced (France and South Korea) and native ranges (Chinese provinces of Yunnan and Zhejiang/Jiangsu, Indonesia, and Vietnam). To enable dataset merging, allele scores of the 21 loci were previously harmonized between laboratories by genotyping ten DNA samples reported in Arca et al. (2015).

Genetic diversity statistics including observed (Na) and effective (Ne) number of alleles, observed (Ho) and expected (He) heterozygosities, and unbiased expected heterozygosity (uHe) were calculated using GENALEX 6.5 (Peakall and Smouse 2012). Allelic richness (A_R), a measure of the number of alleles independent of sample size, per locus and population sample was computed with FSTAT 2.9.4 (Goudet 2001). Differences in average unbiased gene diversity and allelic richness between pairs of samples were assessed by Wilcoxon's signed rank test using R statistic program.

To assess genetic structure and how the 246 individuals grouped with those reported by Arca et al. (2015), as a method for identifying their probable origin, a principal coordinate analysis (PCoA) and a model-based Bayesian clustering analysis were implemented in GENALEX and STRUCTURE 2.3.3 (Pritchard et al. 2000), respectively. In STRUCTURE, the membership proportion (Q-value) of each ancestral cluster (K) was estimated using the admixture ancestry and correlated allele frequency models with the unsupervised option. The program was set up for 750 000 Markov chain Monte Carlo iterations after an initial burn-in of 250 000. To confirm consistency across runs, 20 independent runs for each K (from 1 to 5) were implemented. Q-plots were post-processed online with CLUMPAK (Kopelman et al. 2015). The most probable K was calculated as described by Evanno et al. (2005). To examine the effect of unbalanced sample size in membership partitioning, STRUCTURE was run in six distinct datasets: (i) one dataset, called "Total", containing 416 individuals (246 samples genotyped herein plus the 170 samples genotyped by Arca et al. (2015), sampled from the invaded range in France and South Korea and from the native range in China, Indonesia, and Vietnam); (ii) three datasets, called "Subsets", containing a maximum of 20 individuals per population, which were randomly selected from populations with N > 20 (Portugal, Spain, France, Indonesia, and the Chinese province of Zhejiang/Jiangsu); (iii) one dataset, called "European", containing only the European individuals; (iv) and one dataset, called "Asian", containing only the Asian individuals. Genetic differentiation between populations was assessed by F_{ST} using ARLEQUIN 3.5.2.2 (Excoffier et al. 2005).

Departure from Hardy-Weinberg equilibrium (HWE) was tested for each locus and population using the Hardy-Weinberg Fisher's exact test with the global probability value over all loci calculated using the procedure of Raymond and Rousset (1995a). Heterozygote deficiency and excess were tested at each locus and across loci for each sample using the score test (U-test) according to Rousset and Raymond (1995). These analyses were performed by the Markov chain method with 1 000 dememorization steps, 1 000 batches, and 1 000 iterations per batch, using GENEPOP on the web (Raymond and Rousset 1995b). The presence of a recent bottleneck was assessed in the invasive populations using the Wilcoxon's signed rank test implemented in BOTTLENECK 1.2.0.2 (Piry et al. 1999). This software allows bottleneck testing using three mutational models: infinite alleles model (IAM), stepwise mutational model (SMM) and a combination of the two, named two-phase model (TPM; Di Rienzo et al. 1994). The three models were tested here with TPM run using following combinations of variance (σ) and proportion of SMM: (i) σ=12, SMM=5% (Cristescu et al. 2010), (ii) σ=12, SMM=95% (Piry et al. 1999), (iii) σ=12, SMM=88% (Coster et al. 2019), (iv) σ=30, SMM=20% (Bounas et al. 2018), (v) σ=12, SMM=80% (Sacks and Milburn 2018), (vi) σ=30, SMM=70% (Lalis et al. 2019; Horreo et al. 2019), and (vii) σ=30, SMM=90% (Chen et al. 2018). The significance was assessed by performing 10 000 iterations. Where applicable throughout the analysis, statistical significance levels were adjusted for multiple comparisons using sequential Bonferroni procedure to correct type I error (Rice 1989).

IV. Results

1. Mitochondrial DNA

The diversity of the mitochondrial DNA (mtDNA) was analysed using sequence data of the 642 bp COI barcode region for 38 individuals from Portugal, Spain, and Italy (Figure 5). A simple haplotype, previously named by Arca et al. (2015) as F, was detected for the 38 individuals. To date, F has been the sole haplotype present in Europe (France, Arca et al. 2015, and UK, Budge et al. 2017).

The Maximum Parsimony (MP) phylogenetic tree shown in Figure 6 was generated from 64 COI sequences using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei and Kumar 2000) with search level zero, in which the initial trees were obtained by the random addition of sequences (10 replicates). All nucleotide positions containing gaps and missing data were eliminated. A total of 598 positions remained in the final dataset. The MP tree was obtained with a tree length of 196. MP inferred a consistency index of 0.867 (0.844), a retention index of 0.912 (0.912), and a composite index of 0.791 (0.770) for all sites and for parsimony-informative sites (in parentheses). All European samples are in the same clade, together with two (JQ780450 and JQ780451) out of four samples from the Chinese province of Zhejiang and with a close proximity with one out of three samples from the Chinese province of Yunnan (JQ780457). On the other hand, the three samples from Vietnam and Indonesia were the most divergent (Figure 6).

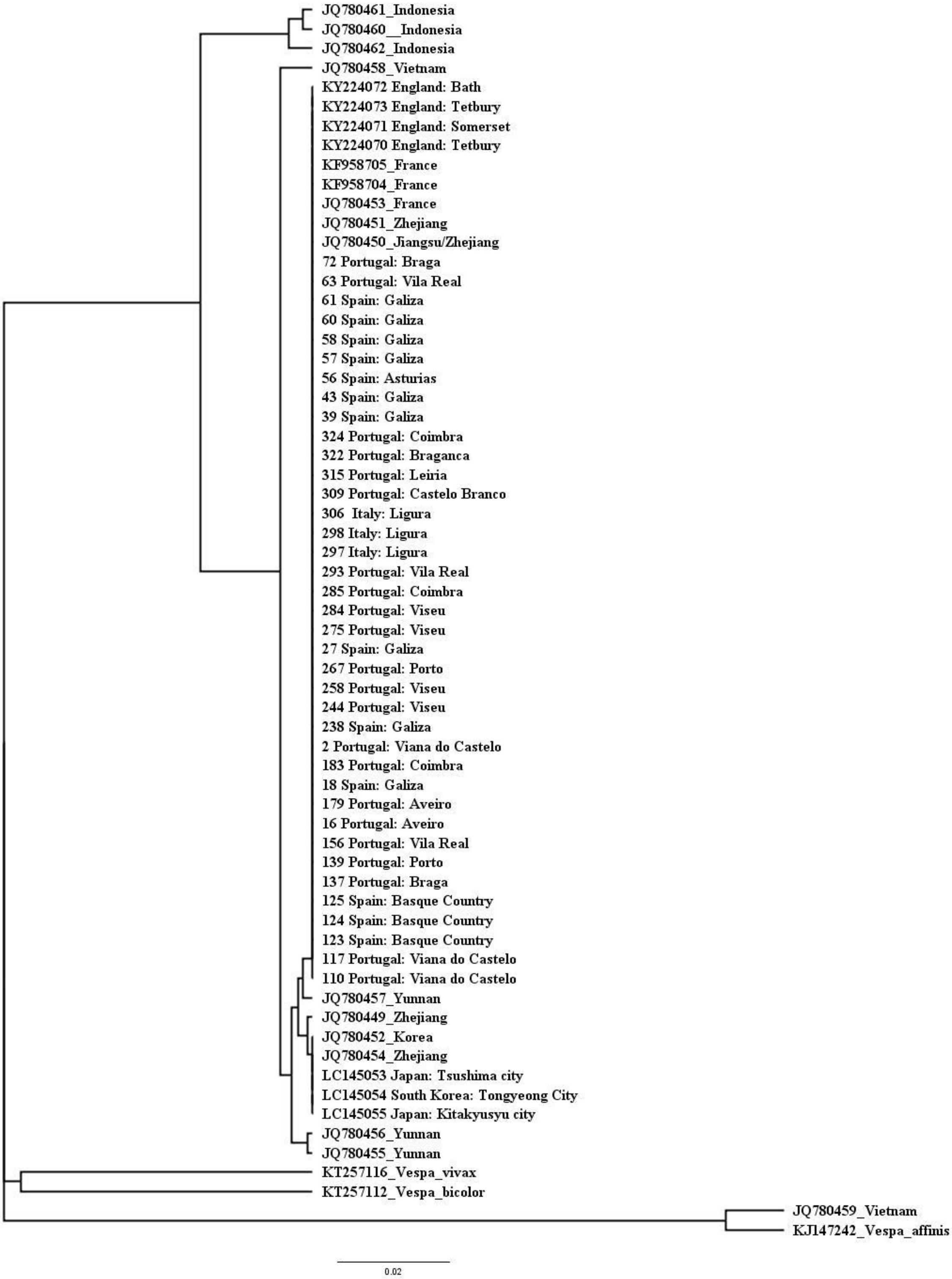

Figure 6: Maximum Parsimony (MP) phylogenetic tree. The MP was obtained with a tree length of 196. The tree is drawn to scale, with branch lengths calculated using the average pathway method (Nei and Kumar 2000) and are in the units of the number of changes over the whole 642-bp sequence. The analysis involved 64 sequences from individuals of the invaded range in Europe and from the native range in Asia

2. Microsatellites

a. Quality control

Of the 21 microsatellites loci screened in this study, due to technical problems, five were excluded from further analyses. While locus R4-26 did not produce repeatable results across laboratories, LIST2004B, R3-115, R1-158, and LIST2003 were unsuccessfully amplified. After removal of the five loci, the total rate of missing data was low (ranging from 0.2% in R1-80 to 14.9% in LIST2018B), particularly in the dataset generated in this study for populations of Portugal, Spain, and Italy, where only the locus VMA-8 showed missing data (0.18%; Table 4).

While GIMLET analysis did not find evidence of genotyping errors, MICRO-CHECKER detected the presence of null alleles in D3-15, LIST2020B, and R1-137 in the population of Portugal, D3-15, LIST2018B, R1-137, D2-185, and VMA-6 in the population of Spain, and none in the Italian population. Given that there was no consistency in the positive results, and Arca et al. (2015) used the same set, all loci were included in further analyses.

b. Genetic diversity

Of the 16 loci that passed quality control, locus R1-77 revealed to be monomorphic for all three populations under study, whereas R1-80 was monomorphic for the Portuguese population and R1-169 for the Italian population. As expected for a founder effect, a dramatic decrease in the number of alleles was observed for European populations. In locus R1-80, for instance, 26 alleles were detected in the populations of the native range, whereas only two were identified in the European populations. In the three European populations, observed number of alleles, effective number of alleles and allelic richness were low varying between 1.000 for the three parameters and 4.000 (LIST2015, Portugal and Spain; LIST2018B, Italy), 2.932 (LIST2018B, Portugal) and 4.000 (LIST2018B, Italy), respectively (Table 4)

Table 4: Summary statistics for the 16 microsatellites, including missing data (MD) in the merged dataset (total) and in the three populations under study (focal), observed number of alleles (Na), effective number of alleles (Ne) and allelic richness (A_R)

Locus	**(%) MD (total)**	**(%) MD (focal)**	**Na (total)**	**Na (Portugal)**	**Na (Spain)**	**Na (Italy)**	**Ne (Portugal)**	**Ne (Spain)**	**Ne (Italy)**	**A_R (Portugal)**	**A_R (Spain)**	**A_R (Italy)**
R1-36	0.50	0.00	10	2.000	2.000	2.000	1.613	1.887	1.541	1.999	2.000	2.000
R1-169	1.90	0.00	7	2.000	2.000	1.000	1.915	1.953	1.000	2.000	2.000	1.000
R4-114	0.50	0.00	11	2.000	3.000	2.000	1.856	2.073	1.984	2.000	2.431	2.000
D3-15	2.20	0.00	10	3.000	3.000	2.000	1.049	1.783	1.541	1.472	2.950	2.000
LIST2015	0.50	0.00	13	4.000	4.000	2.000	1.765	2.809	2.000	2.317	3.240	2.000
LIST2020B	5.00	0.00	13	2.000	3.000	2.000	1.021	1.250	1.862	1.213	2.175	2.000
R4-33	2.20	0.00	12	2.000	2.000	2.000	1.808	1.670	1.984	2.000	2.000	2.000
R1-77	0.50	0.00	8	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LIST2018B	14.90	0.00	13	3.000	3.000	4.000	2.932	2.772	2.373	2.999	2.998	4.000
R1-137	1.20	0.00	16	3.000	3.000	2.000	1.066	2.090	1.766	1.605	2.904	2.000
D2-185	1.00	0.00	9	2.000	2.000	2.000	1.957	1.923	1.936	2.000	2.000	2.000
VMA-8	13.90	0.18	16	3.000	2.000	2.000	2.003	1.923	1.424	2.113	2.000	2.000
R1-80	0.20	0.00	26	1.000	2.000	2.000	1.000	1.442	1.095	1.000	1.995	2.000
R1-75	0.50	0.00	5	3.000	3.000	3.000	1.975	1.776	2.180	2.113	2.762	3.000
R4-100	1.00	0.00	17	2.000	2.000	2.000	1.819	1.499	1.862	2.000	1.998	2.000
VMA-6	13.20	0.00	3	2.000	2.000	2.000	1.038	1.697	1.936	1.344	2.000	2.000

Allele frequencies, observed number of alleles, effective number of alleles, number of private alleles, allelic richness, and heterozygosities are shown, for each locus and population of the invaded and native areas, in Table 5. As expected, the microsatellite variability was much lower in the invaded European countries than in the native distributional area in Asia. The mean number of observed/effective alleles (Na/Ne) ranged from 2.06 (Italy)/1.61 (Portugal) to 4.13/2.41 (France) in Europe, and 2.81/2.19 (South Korea) to 7.19/5.01 (Yunnan) in Asia. The number of private alleles was high in the Asian populations, varying between five (Vietnam) and 16 (Yunnan), contrasting with the sole private allele (locus LIST2020B, allele 183) detected in Spain. In Europe, as a whole, the number of private alleles observed was five (R4-33, allele 205 and 209; LIST2018, allele 183 and 121; VMA-8, allele 251).

Allelic richness across loci and populations was significantly lower (P-value ≤ 0.04372; Wilcoxon's signed rank test; Table S2) in the European (mean = 2.176) than in the native Asian populations (mean = 4.345). Within Europe, a significantly higher (P-value ≤ 0.0020, Wilcoxon's signed rank test; Table S2) allelic richness was found for the French (2.803) than for the Spanish (2.179), Italian (1.962), or Portuguese (1.760) populations.

Observed and expected heterozygosities (Ho/He) across loci were similar among the three focal populations, ranging from 0.30/0.31 (Portugal) to 0.37 (Italy)/0.42 (Spain), and lower than those exhibited by the invasive population of France (0.48/0.55) and of the native range (varying between 0.43 in Indonesia/0.54 in Vietnam to 0.74/0.77 in the Chinese province of Yunnan). Genetic diversity, expressed by unbiased expected heterozygosity (uHe), was significantly lower (P-value ≤ 0.0034, Wilcoxon's signed rank test; Table S2) in Portugal (0.31), Italy (0.39), and Spain (0.43) than in France (0.55) and in the native countries (varying between 0.57 in Vietnam to 0.79 in the Chinese province of Yunnan).

Table 5: Allele frequencies, observed number of alleles (Na), effective number of alleles (Ne), allelic richness (A_R), number of private alleles (NPa), missing data (MD), observed heterozygosity (Ho), expected heterozygosity (He) and unbiased expected heterozygosity (uHe) across loci and population

Locus R1-36	**Allele** (bp)	**Portugal** (N=190)	**Spain** (N=45)	**Italy** (N=11)	**France** (N=83)	**South Korea** (N=8)	**Vietnam** (N=8)	**Indonesia** (N=21)	**China Yunnan** (N=18)	**China Zhejiang/ Jiangsu** (N=30)
	97				0.006	0.500		0.095	0.056	0.367
	99	0.745	0.622	0.773	0.699		0.063	0.429	0.167	0.233
	101							0.071	0.250	0.067
	103					0.188			0.056	0.167
	105					0.313			0.083	0.133
	107	0.255	0.378	0.227	0.295		0.500	0.095	0.194	0.033
	111						0.438	0.024	0.167	
	113							0.143	0.028	
	115							0.071		
	117							0.071		
Na		2.000	2.000	2.000	3.000	3.000	3.000	8.000	8.000	6.000
Ne		1.613	1.887	1.541	1.738	2.612	2.246	4.200	5.891	4.167
A_R		1.973	1.998	1.990	2.060	2.993*	2.750	5.622	5.980	4.700*
Ho		0.374	0.489	0.273	0.410	0.625	0.500	0.524	0.833	0.567
He		0.380	0.470	0.351	0.425	0.617	0.555	0.762	0.830	0.760
uHe		0.381	0.475	0.368	0.427	0.658	0.592	0.780	0.854	0.773

Locus R1-169	**Allele** (bp)	**Portugal** (N=190)	**Spain** (N=45)	**Italy** (N=11)	**France** (N=76)	**South Korea** (N=8)	**Vietnam** (N=8)	**Indonesia** (N=21)	**China Yunnan** (N=20)	**China Zhejiang/ Jiangsu** (N=29)
	148								0.225	
	150					0.438			0.175	0.138
	152								0.125	0.017
	156						0.875	0.048	0.175	0.034
	158	0.395	0.422	1.000	0.757	0.563		0.595	0.175	0.534
	161						0.125	0.357	0.025	0.052
	163	0.605	0.578		0.243				0.100	0.224
Na		2.000	2.000	1.000	2.000	2.000	2.000	3.000	7.000	6.000
Ne		1.915	1.953	1.000	1.583	1.969	1.280	2.066	5.926	2.785
A_R		1.998	1.999	1.000	1.970	2.000*	1.950	2.493	5.711	3.920*
Ho		0.495	0.578	0.000	0.276	0.625	0.250	0.238	0.850	0.690
He		0.478	0.488	0.000	0.368	0.492	0.219	0.516	0.831	0.641
uHe		0.479	0.493	0.000	0.371	0.525	0.233	0.528	0.853	0.652

Table 5: Continued

Locus R4-114	**Allele (bp)**	**Portugal** (N=190)	**Spain** (N=45)	**Italy** (N=11)	**France** (N=82)	**South Korea** (N=8)	**Vietnam** (N=8)	**Indonesia** (N=21)	**China Yunnan** (N=20)	**China Zhejiang/ Jiangsu** (N=29)
	118				0.012		0.438		0.375	
	122								0.050	
	124								0.125	0.017
	126				0.030		0.313	0.381	0.100	0.052
	128	0.361	0.444	0.545	0.457	0.063	0.125	0.381	0.100	0.121
	130					0.438		0.214	0.250	0.362
	132					0.438	0.125	0.024		0.172
	134		0.022		0.220	0.063				0.121
	138	0.639	0.533	0.455	0.280					0.034
	142									0.103
	148									0.017
Na		2.000	3.000	2.000	5.000	4.000	4.000	4.000	6.000	9.000
Ne		1.856	2.073	1.984	2.967	2.560	3.122	2.970	4.145	4.875
AR		1.996	2.250	2.000	3.399	3.500*	3.900	3.252	4.902	5.628*
Ho		0.416	0.444	0.364	0.646	0.750	0.625	0.238	0.850	0.759
He		0.461	0.518	0.496	0.663	0.609	0.680	0.663	0.759	0.795
uHe		0.462	0.523	0.519	0.667	0.650	0.725	0.679	0.778	0.809

Locus D3-15	**Allele (bp)**	**Portugal** (N=190)	**Spain** (N=45)	**Italy** (N=11)	**France** (N=81)	**South Korea** (N=8)	**Vietnam** (N=8)	**Indonesia** (N=18)	**China Yunnan** (N=20)	**China Zhejiang/ Jiangsu** (N=26)
	157									0.038
	160	0.011	0.122	0.227	0.216			0.028	0.175	0.038
	162				0.006	0.188		0.917		0.019
	164				0.062	0.063		0.056	0.325	0.135
	166	0.976	0.722		0.383	0.688			0.175	0.365
	168					0.063			0.050	0.154
	170	0.013	0.156	0.773	0.333		0.125			0.058
	172						0.688		0.100	0.135
	174						0.188		0.175	0.038
	180									0.019
Na		3.000	3.000	2.000	5.000	4.000	3.000	3.000	6.000	10.000
Ne		1.049	1.783	1.541	3.246	1.939	1.910	1.185	4.762	4.952
AR		1.270	2.700	1.990	3.565	3.493*	2.943	1.895	5.098	5.867*
Ho		0.026	0.244	0.455	0.691	0.500	0.375	0.167	0.900	0.846
He		0.047	0.439	0.351	0.692	0.484	0.477	0.156	0.790	0.798
uHe		0.047	0.444	0.368	0.696	0.517	0.508	0.160	0.810	0.814

Table 5: Continued

Locus **LIST2015**	**Allele** (bp)	**Portugal** (N=190)	**Spain** (N=45)	**Italy** (N=11)	**France** (N=83)	**South Korea** (N=7)	**Vietnam** (N=8)	**Indonesia** (N=21)	**China Yunnan** (N=20)	**China Zhejiang/ Jiangsu** (N=29)
	169				0.006					
	178	0.013	0.189	0.500	0.355	0.500			0.075	0.052
	180	0.289	0.389	0.500	0.343	0.429		0.262	0.375	0.207
	182				0.012			0.310	0.125	0.259
	184							0.095	0.175	0.017
	186	0.695	0.411		0.259		0.188	0.048	0.100	0.103
	188				0.006	0.071		0.095		0.103
	190		0.011				0.750	0.119	0.100	0.086
	192	0.003			0.012		0.063	0.024	0.025	0.017
	194							0.048		
	196									0.017
	198				0.006				0.025	0.017
	200									0.121
Na		4.000	4.000	2.000	8.000	3.000	3.000	8.000	8.000	11.000
Ne		1.765	2.809	2.000	3.208	2.279	1.662	4.955	4.678	6.371
A_R		2.165	3.064	2.000	3.464	2.857*	2.743	5.601	5.607	6.335*
Ho		0.432	0.533	0.455	0.530	0.286	0.375	0.524	0.750	0.931
He		0.433	0.644	0.500	0.688	0.561	0.398	0.798	0.786	0.843
uHe		0.435	0.651	0.524	0.692	0.604	0.425	0.818	0.806	0.858

Locus **LIST2020B**	**Allele** (bp)	**Portugal** (N=190)	**Spain** (N=45)	**Italy** (N=11)	**France** (N=82)	**South Korea** (N=6)	**Vietnam** (N=8)	**Indonesia** (N=14)	**China Yunnan** (N=19)	**China Zhejiang/ Jiangsu** (N=20)
	181								0.053	0.150
	183		0.011							
	185				0.024		0.688	0.071	0.026	
	187				0.085		0.188	0.071	0.132	0.075
	189	0.989	0.889	0.636	0.598	0.667				0.275
	191						0.063	0.107		
	193				0.018	0.333		0.286	0.105	0.200
	195	0.011	0.100	0.364	0.268		0.063	0.036	0.026	0.275
	197							0.036	0.132	0.025
	199				0.006			0.071	0.132	
	203							0.107	0.368	
	213							0.107		
	215							0.107	0.026	
Na		2.000	3.000	2.000	6.000	2.000	4.000	10.000	9.000	6.000
Ne		1.021	1.250	1.862	2.287	1.800	1.939	6.877	4.912	4.545
A_R		1.121	1.875	2.000	3.193	2.000*	3.493	7.217	5.888	4.811*
Ho		0.011	0.133	0.364	0.549	0.667	0.500	0.786	0.632	1.000
He		0.021	0.200	0.463	0.563	0.444	0.484	0.855	0.796	0.780
uHe		0.021	0.202	0.485	0.566	0.485	0.517	0.886	0.818	0.800

Table 5: Continued

Locus R4-33	**Allele** (bp)	**Portugal** (N=190)	**Spain** (N=45)	**Italy** (N=11)	**France** (N=81)	**South Korea** (N=8)	**Vietnam** (N=8)	**Indonesia** (N=18)	**China Yunnan** (N=20)	**China Zhejiang/ Jiangsu** (N=26)
	157									0.038
	160				0.216			0.028	0.175	0.038
	162				0.006	0.188		0.917		0.019
	164				0.062	0.063		0.056	0.325	0.135
	166				0.383	0.688			0.175	0.365
	168					0.063			0.050	0.154
	170				0.333		0.125			0.058
	172						0.688		0.100	0.135
	174						0.188		0.175	0.038
	180									0.019
	205	0.663	0.722	0.455						
	209	0.337	0.278	0.545						
Na		2.000	2.000	2.000	5.000	4.000	3.000	3.000	6.000	10.000
Ne		1.808	1.670	1.984	3.246	1.939	1.910	1.185	4.762	4.952
A_R		1.993	1.985	2.000	3.565	3.493*	2.943	1.895	5.098	5.867*
Ho		0.463	0.422	0.727	0.691	0.500	0.375	0.167	0.900	0.846
He		0.447	0.401	0.496	0.692	0.484	0.477	0.156	0.790	0.798
uHe		0.448	0.406	0.519	0.696	0.517	0.508	0.160	0.810	0.814

Locus R1-77	**Allele** (bp)	**Portugal** (N=190)	**Spain** (N=45)	**Italy** (N=11)	**France** (N=82)	**South Korea** (N=8)	**Vietnam** (N=8)	**Indonesia** (N=21)	**China Yunnan** (N=19)	**China Zhejiang/ Jiangsu** (N=30)
	245							0.024	0.053	
	247							0.119	0.000	0.017
	249							0.071	0.132	
	251							0.381	0.079	0.250
	253	1.000	1.000	1.000	0.939	0.125	0.563	0.357	0.447	0.250
	255				0.061	0.563	0.438	0.048	0.184	0.200
	257								0.105	0.167
	259					0.313				0.117
Na		1.000	1.000	1.000	2.000	3.000	2.000	6.000	6.000	6.000
Ne		1.000	1.000	1.000	1.129	2.327	1.969	3.392	3.684	4.839
A_R		1	1	1	1.543	2.95*	2	4.257	4.844	4.832*
Ho		0.000	0.000	0.000	0.000	0.875	0.375	0.476	0.737	0.767
He		0.000	0.000	0.000	0.115	0.570	0.492	0.705	0.729	0.793
uHe		0.000	0.000	0.000	0.115	0.608	0.525	0.722	0.748	0.807

Table 5: Continued

Locus LIST2018B	**Allele** (bp)	**Portugal** (N=190)	**Spain** (N=45)	**Italy** (N=11)	**France** (N=75)	**South Korea** (N=0)	**Vietnam** (N=8)	**Indonesia** (N=6)	**China Yunnan** (N=19)	**China Zhejiang/ Jiangsu** (N=0)
	113	0.400	0.456	0.409	0.480				0.553	
	121	0.276	0.222	0.045	0.147					
	125								0.132	
	127						0.250		0.132	
	131								0.079	
	133								0.105	
	137	0.324	0.322	0.500	0.353		0.500			
	139			0.045	0.007		0.250			
	143							0.333		
	145				0.013			0.083		
	147							0.250		
	153							0.250		
	161							0.083		
Na		3.000	3.000	4.000	5.000	0.000	3.000	5.000	5.000	0.000
Ne		2.932	2.772	2.373	2.653	0.000	2.667	4.000	2.798	0.000
A_R		2.970	2.954	3.091	3.092	-	2.999	5.000	4.227	-
Ho		0.653	0.467	0.636	0.493		0.500	0.833	0.632	
He		0.659	0.639	0.579	0.623		0.625	0.750	0.643	
uHe		0.661	0.646	0.606	0.627		0.667	0.818	0.660	

Locus R1-137	**Allele** (bp)	**Portugal** (N=190)	**Spain** (N=45)	**Italy** (N=11)	**France** (N=80)	**South Korea** (N=7)	**Vietnam** (N=8)	**Indonesia** (N=21)	**China Yunnan** (N=19)	**China Zhejiang/ Jiangsu** (N=30)
	168							0.190		
	170							0.119		
	172					0.286		0.429	0.026	0.067
	174							0.071	0.026	
	176							0.167		
	180							0.024	0.026	0.017
	182	0.016	0.289		0.425		0.125		0.132	0.050
	184						0.063		0.184	0.033
	186	0.968	0.622	0.682	0.369	0.286	0.125		0.105	0.117
	188						0.250		0.105	0.083
	190	0.016	0.089	0.318	0.206		0.188		0.184	0.200
	192					0.286	0.125			0.200
	194								0.026	0.017
	196					0.143	0.125		0.105	0.183
	198									0.033
	202								0.079	
Na		3.000	3.000	2.000	3.000	4.000	7.000	6.000	11.000	11.000
Ne		1.066	2.090	1.766	2.784	3.769	6.095	3.737	7.848	6.950
A_R		1.352	2.686	1.999	2.940	3.989^*	6.542	4.639	7.112	6.551^*
Ho		0.032	0.333	0.636	0.538	0.714	0.750	0.524	0.842	0.833
He		0.062	0.521	0.434	0.641	0.735	0.836	0.732	0.873	0.856
uHe		0.062	0.527	0.455	0.645	0.791	0.892	0.750	0.896	0.871

Table 5: Continued

Locus D2-185	**Allele** (bp)	**Portugal** (N=190)	**Spain** (N=45)	**Italy** (N=11)	**France** (N=80)	**South Korea** (N=7)	**Vietnam** (N=8)	**Indonesia** (N=21)	**China Yunnan** (N=20)	**China Zhejiang/ Jiangsu** (N=30)
	208	0.426	0.600	0.591	0.588	0.071	0.063	0.048	0.250	0.017
	210					0.214			0.225	0.217
	212					0.286		0.095	0.125	0.467
	214	0.574	0.400	0.409	0.406	0.357	0.625	0.405	0.175	0.117
	216				0.006	0.071		0.143	0.125	0.117
	218							0.310		0.050
	222						0.125		0.075	
	224						0.188			0.017
	228								0.025	
Na		2.000	2.000	2.000	3.000	5.000	4.000	5.000	7.000	7.000
Ne		1.957	1.923	1.936	1.960	3.769	2.246	3.432	5.517	3.390
A_R		1.999	1.999	2.000	2.074	4.714*	3.693	4.132	5.565	4.476*
Ho		0.516	0.311	0.455	0.550	0.857	0.500	0.476	0.950	0.633
He		0.489	0.480	0.483	0.490	0.735	0.555	0.709	0.819	0.705
uHe		0.490	0.485	0.506	0.493	0.791	0.592	0.726	0.840	0.717

Locus VMA-8	**Allele** (bp)	**Portugal** (N=189)	**Spain** (N=45)	**Italy** (N=11)	**France** (N=78)	**South Korea** (N=0)	**Vietnam** (N=8)	**Indonesia** (N=6)	**China Yunnan** (N=19)	**China Zhejiang/ Jiangsu** (N=2)
	233								0.079	
	243							0.167		
	245							0.167		0.750
	247							0.083	0.079	
	249				0.019			0.083	0.079	0.250
	251	0.450	0.400	0.818	0.186					
	255							0.083	0.105	
	257								0.237	
	259							0.250	0.132	
	261						0.063			
	263				0.026		0.250		0.132	
	265				0.115		0.438	0.083		
	267	0.545	0.600	0.182	0.647		0.063		0.053	
	269	0.005			0.006		0.063		0.105	
	271						0.063			
	273						0.063	0.083		
Na		3.000	2.000	2.000	6.000	0.000	7.000	8.000	9.000	2.000
Ne		2.003	1.923	1.424	2.136	0.000	3.657	6.545	7.443	1.600
A_R		2.062	1.999	1.971	3.275	-	5.749	8.000	6.927	-
Ho		0.476	0.533	0.182	0.462		1.000	0.833	0.947	0.500
He		0.501	0.480	0.298	0.532		0.727	0.847	0.866	0.375
uHe		0.502	0.485	0.312	0.535		0.775	0.924	0.889	0.500

Table 5: Continued

Locus R1-80	**Allele** (bp)	**Portugal** (N=190)	**Spain** (N=45)	**Italy** (N=11)	**France** (N=82)	**South Korea** (N=8)	**Vietnam** (N=8)	**Indonesia** (N=21)	**China Yunnan** (N=20)	**China Zhejiang/ Jiangsu** (N=30)
	96					0.250				0.100
	98									0.017
	100									0.133
	102								0.100	0.067
	104					0.313				0.050
	106									0.150
	108		0.189	0.045	0.341			0.071	0.075	0.100
	110	1.000	0.811	0.955	0.659			0.119	0.225	0.267
	116									0.017
	120							0.548		
	132								0.175	0.017
	134								0.025	
	136								0.100	
	138					0.188		0.071	0.025	0.033
	140						0.188			
	142						0.125	0.119	0.075	
	144									0.017
	148						0.063			
	152						0.250	0.024		
	154							0.048		
	156						0.188		0.050	
	158						0.188			
	160								0.025	
	162								0.075	0.017
	166								0.050	
	172					0.250				0.017
Na		1.000	2.000	2.000	2.000	4.000	6.000	7.000	12.000	14.000
Ne		1.000	1.442	1.095	1.817	3.879	5.333	2.930	8.000	7.087
A_R		1.000	1.933	1.545	1.995	3.992*	5.678	4.738	7.399	6.892*
Ho		0.000	0.200	0.091	0.439	0.500	1.000	0.333	0.700	0.700
He		0.000	0.306	0.087	0.450	0.742	0.813	0.659	0.875	0.859
uHe		0.000	0.310	0.091	0.452	0.792	0.867	0.675	0.897	0.873

Table 5: Continued

Locus R1-75	**Allele** (bp)	**Portugal** (N=190)	**Spain** (N=45)	**Italy** (N=11)	**France** (N=83)	**South Korea** (N=7)	**Vietnam** (N=8)	**Indonesia** (N=21)	**China Yunnan** (N=19)	**China Zhejiang/ Jiangsu** (N=30)
	144	0.005	0.056	0.318	0.367	0.571	0.813	0.333	0.447	0.617
	146					0.286			0.158	0.150
	152	0.421	0.233	0.591	0.181		0.125	0.452	0.395	0.133
	154							0.119		
	156	0.574	0.711	0.091	0.452	0.143	0.063	0.095		0.100
Na		3.000	3.000	3.000	3.000	3.000	3.000	4.000	3.000	4.000
Ne		1.975	1.776	2.180	2.689	2.333	1.471	2.950	2.625	2.323
A_R		2.061	2.487	2.804	2.913	2.989^*	2.700	3.585	2.916	3.494^*
Ho		0.532	0.400	0.273	0.602	0.571	0.250	0.429	0.526	0.733
He		0.494	0.437	0.541	0.628	0.571	0.320	0.661	0.619	0.569
uHe		0.495	0.442	0.567	0.632	0.615	0.342	0.677	0.636	0.579

Locus R4-100	**Allele** (bp)	**Portugal** (N=190)	**Spain** (N=45)	**Italy** (N=11)	**France** (N=82)	**South Korea** (N=8)	**Vietnam** (N=8)	**Indonesia** (N=21)	**China Yunnan** (N=19)	**China Zhejiang/ Jiangsu** (N=28)
	158									0.054
	160								0.342	
	162								0.053	
	164							0.048		0.018
	166					0.313		0.690	0.158	0.143
	168							0.238	0.026	0.036
	170							0.024	0.158	
	172						0.438		0.079	0.054
	174						0.125		0.026	
	176						0.438			0.143
	178				0.006	0.250				0.214
	180	0.658	0.789	0.636	0.427					0.071
	182				0.159	0.250			0.026	0.125
	184	0.342	0.211	0.364	0.366					0.054
	186				0.043					0.071
	188					0.188				0.018
	198								0.132	
Na		2.000	2.000	2.000	5.000	4.000	3.000	4.000	9.000	12.000
Ne		1.819	1.499	1.862	2.915	3.879	2.510	1.865	5.121	8.124
A_R		1.994	1.953	2.000	3.371	3.992^*	2.950	2.760	5.877	7.189^*
Ho		0.379	0.244	0.545	0.280	0.750	0.375	0.238	0.316	0.714
He		0.450	0.333	0.463	0.657	0.742	0.602	0.464	0.805	0.877
uHe		0.451	0.337	0.485	0.661	0.792	0.642	0.475	0.826	0.893

Table 5: Continued

Locus VMA-6	**Allele** (bp)	**Portugal** (N=190)	**Spain** (N=45)	**Italy** (N=11)	**France** (N=81)	**South Korea** (N=0)	**Vietnam** (N=8)	**Indonesia** (N=6)	**China Yunnan** (N=20)	**China Zhejiang/ Jiangsu** (N=0)
	240				0.043			0.083	0.025	
	242	0.018	0.289	0.591	0.475		0.188	0.750	0.600	
	244	0.982	0.711	0.409	0.481		0.813	0.167	0.375	
Na		2.000	2.000	2.000	3.000	0.000	2.000	3.000	3.000	0.000
Ne		1.038	1.697	1.936	2.176	0.000	1.438	1.674	1.995	0.000
AR		1.203	1.988	2.000	2.422	-	1.993	3.000	2.299	-
Ho		0.026	0.133	0.455	0.506		0.125	0.167	0.550	
He		0.036	0.411	0.483	0.540		0.305	0.403	0.499	
uHe		0.036	0.415	0.506	0.544		0.325	0.439	0.512	

	Portugal	**Spain**	**Italy**	**France**	**South Korea**	**Vietnam**	**Indonesia**	**China Yunnan**	**China Zhejiang/ Jiangsu**
Na	2.31	2.44	2.06	4.13	2.81	3.69	5.44	7.19	7.13
± SE	±0.2	±0.18	±0.17	±0.44	±0.4	±0.41	±0.56	±0.63	±1.05
Ne	1.61	1.85	1.72	2.41	2.19	2.59	3.37	5.01	4.19
± SE	±0.14	±0.12	±0.1	±0.16	±0.33	±0.34	±0.42	±0.44	±0.6
AR	1.760	2.179	1.962	2.803	5.428*	3.439	4.255	5.341	5.538*
NPa**	0	1	0	0	0	5	15	16	15
MD (%)	0.03	0.00	0.00	2.72	23.13	0.00	17.00	2.70	22.63
Ho	0.30	0.34	0.37	0.48	0.51	0.49	0.43	0.74	0.66
± SE	±0.06	±0.04	±0.06	±0.04	±0.07	±0.06	±0.06	±0.04	±0.07
He	0.31	0.42	0.38	0.55	0.49	0.54	0.61	0.77	0.65
± SE	±0.06	±0.04	±0.05	±0.04	±0.07	±0.04	±0.05	±0.03	±0.07
uHe	0.31	0.43	0.39	0.55	0.52	0.57	0.64	0.79	0.67
± SE	±0.06	±0.04	±0.05	±0.04	±0.07	±0.05	±0.06	±0.03	±0.07

*Allelic richness was estimated from only 13 loci, given the high rate of missing data for the three excluded loci

**Private alleles with a frequency > 0.01

c. Population structure and differentiation

Genetic structure was inferred from the microsatellites using two approaches: the model-free principal coordinate analysis (PCoA) and the Bayesian model-based STRUCTURE. The PCoA results show that 59.17% of the variation was explained by the first two components (25.31% and 33.86% for PCo1 and 2, respectively, Figure 7). Asian and European populations are genetically distinct with individuals from Indonesia, China (Zhejiang/Jiangsu), and South Korea and individuals from Iberia being placed in the two extremes of the variation spectrum. Most Portuguese (183) and some individuals of the Spanish (24) population form a tight cluster, which diverge from the other groups. All Italian and some Spanish (21) and Portuguese (7) individuals overlap with those from France.

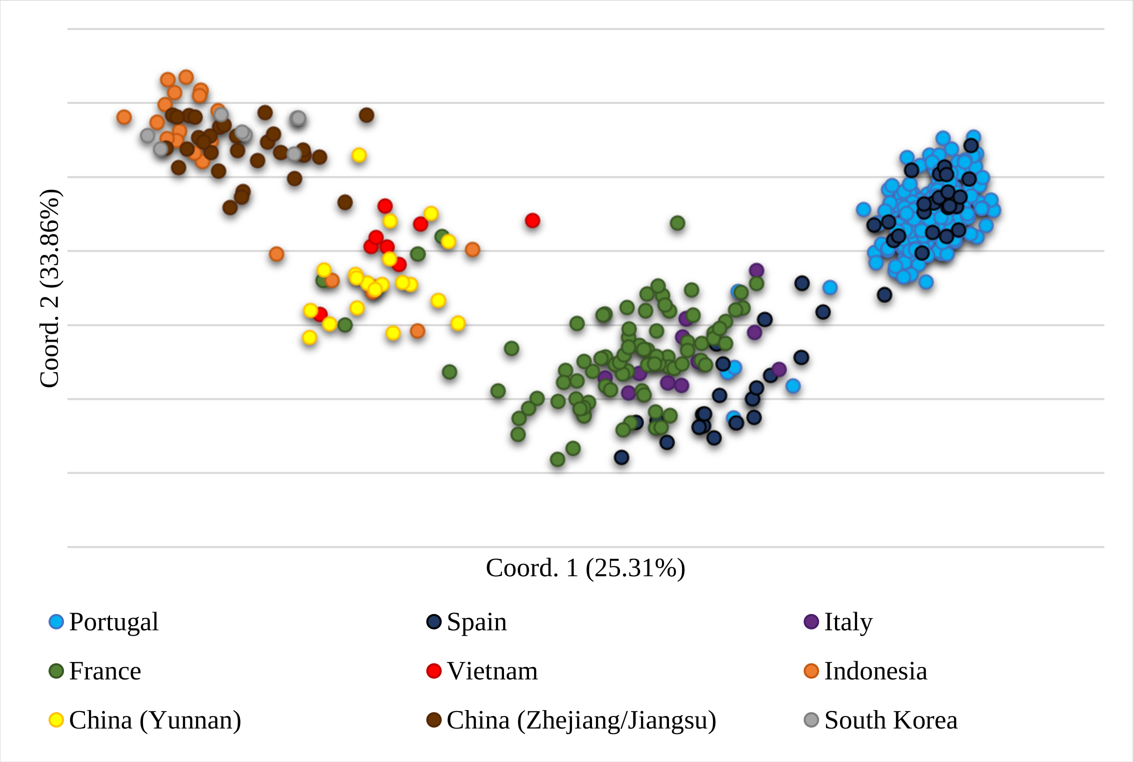

Figure 7: Principle coordinate analysis (PCoA) of *V. v. nigrithorax* (genetic clusters) estimated using GENALEX 6.503 (Peakall and Smouse 2012). The distance metric representation of the genetic clustering shows that 25.31% of the variation is explained by the first axis and 33.86% by the second axis

The Bayesian analysis using STRUCTURE were performed for six datasets, combining a variable number of individuals of distinct geographical origins. These analyses clearly indicated the presence of substructure not only in the native distributional area of *V. v. nigrithorax* in Asia, as expected, but also in the introduced range in Europe (Figures 8-12). Assignment inferred from the "Total" dataset showed that, at the optimal K = 2, as identified by Evanno's test, all individuals from Europe were assigned to the blue cluster, although three of the 83 French individuals exhibited significant levels of Asian ancestry (Q-values ≥ 0.4 in the orange cluster). At K = 3, all French individuals clustered together (purple) with all Italian individuals (11), and with some individuals from Spain (19 out of 45) and Portugal (6 out of 190). The blue cluster grouped most individuals from Portugal and Spain (Figure 8). The spatial distribution of the Iberian individuals is seen in Figure 9. In the Portuguese population, three out of six were collected in the north of the country, while the other three are from the centre region. The Spanish individuals are well distributed in Galicia, and three out of 19 are from the Basque Country.

The "Total" dataset has a markedly uneven sample size across populations, and some studies (Rosenberg et al. 2005; Kalinowski 2011) showed that this may affect the membership partitioning pattern. To test this effect, three subsets of 20 randomly selected individuals were constructed for the populations with a sample size ≥ 20. STRUCTURE analysis showed that, at K = 2, membership partitioning inferred from the three subsets (Figure 10) is similar to that of the "Total" dataset, with European and Asian individuals forming two distinct clusters (Figure 8). However, at K = 3, structure patterns inferred from the different datasets were incongruent, with partitioning occurring earlier in Asia than in Europe (Figure 10). Indonesian individuals formed a distinct cluster (purple) and the individuals from Vietnam showed significant levels of the Indonesian ancestry (Q-values ≥ 0.4 in the purple cluster). At K = 4, the populations from Vietnam and the Chinese province of Yunnan were clustered together (green). The native population from Zhejiang/Jiangsu (China) and the invasive individuals from South Korea were assigned to the Asian cluster (blue). These results showed that the number of individuals seemingly affect the membership partitioning pattern.

Genetic structure inferred from the "European" dataset showed that, at K = 2, all individuals from France, Italy, and some individuals from Spain (19 out of 45) and Portugal (6 out of 190) are clustered together (Q-values ≥ 0.7 in the orange cluster), consistent with the patterns obtained with the “Total” dataset (Figure 11). When K = 3, STRUCTURE was unable to assign individuals to a new cluster. Due to the high levels of missing data observed in LIST2018B (55.3%), VMA-8 (54%) and VMA-6 (54.3%), clustering patterns were inferred from only 13 loci in the “Asian” dataset (Figure 12). Indonesian and Vietnamese individuals were the first to separate from the other Asian populations (orange). At K = 3, Vietnamese and Chinese (Yunnan) individuals clustered together and separate at K = 5, with Vietnamese individuals forming a single cluster (pink).

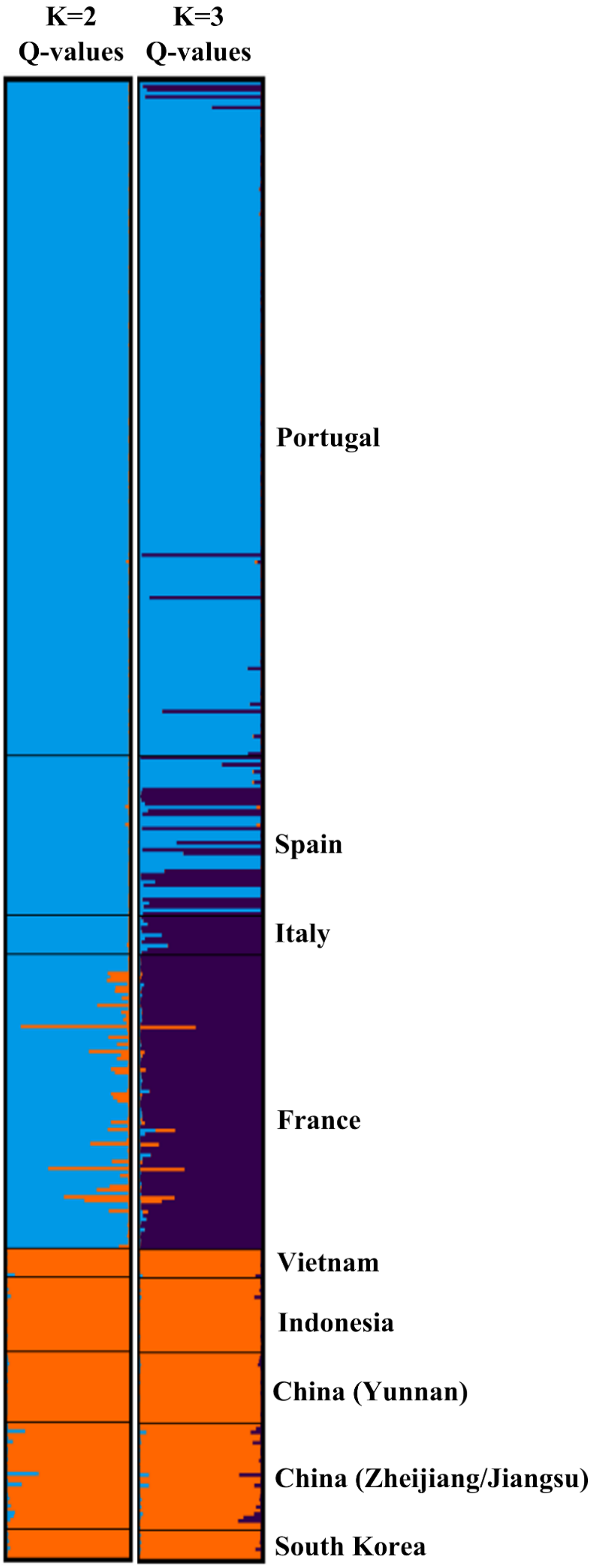

Figure 8: Total dataset. STRUCTURE plot showing the membership partitioning into two (K = 2) and three (K = 3) clusters. Each individual is represented by a horizontal bar. Horizontal black lines separate individuals from different populations

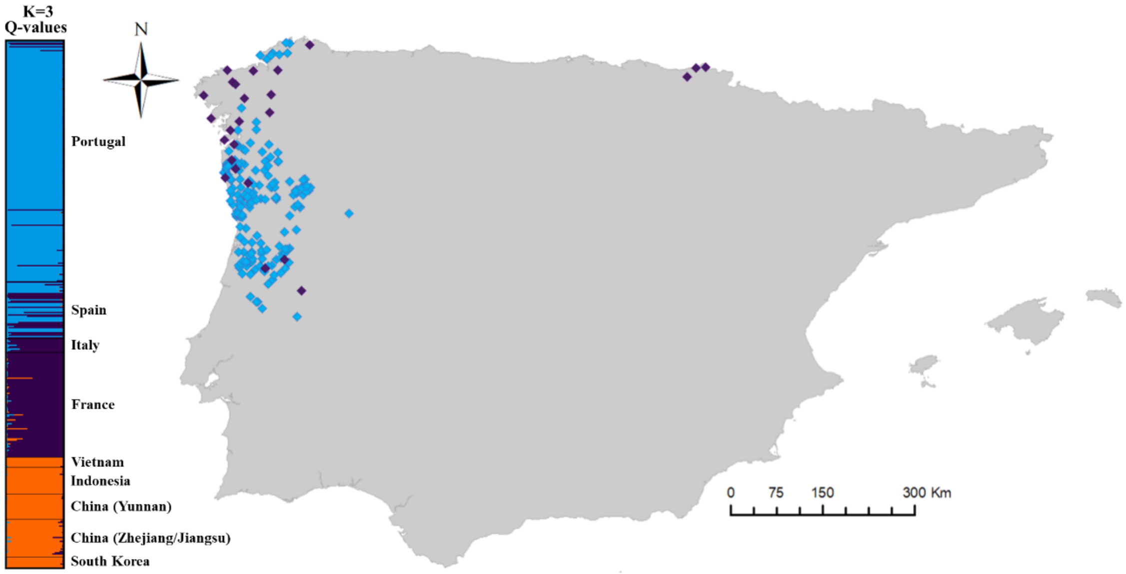

Figure 9: Spatial distribution of the Iberian individuals. The dots colour is coincident with the STRUCTURE analysis: the purple dots corresponds to the individuals with Q-values ≥ 0.7 of the French component (migrants)

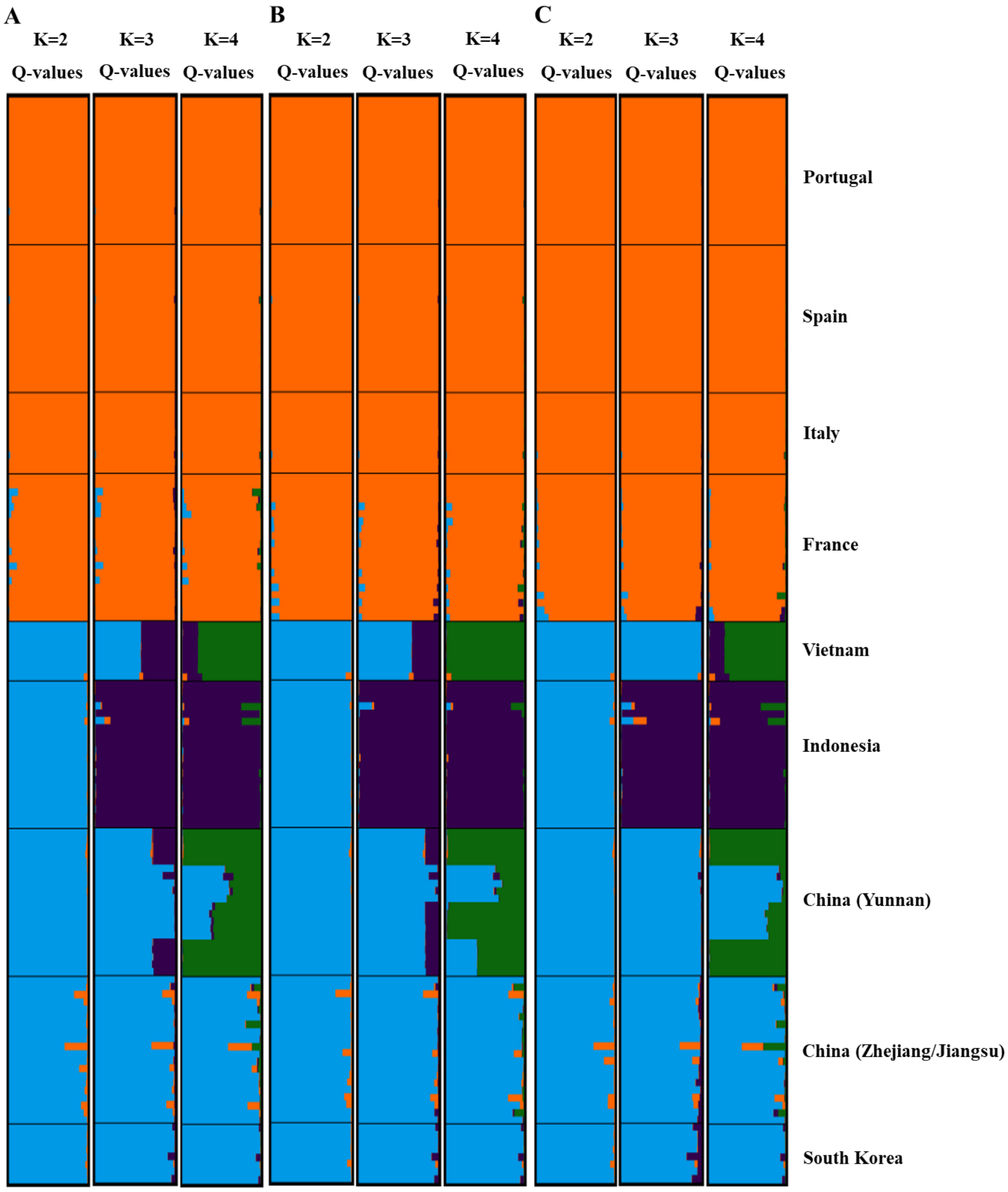

Figure 10: Subsets dataset. STRUCTURE plot showing the membership partitioning into two (K = 2), three (K = 3), and four (K = 4) clusters. Each individual is represented by a horizontal bar. Horizontal black lines separate individuals from different populations

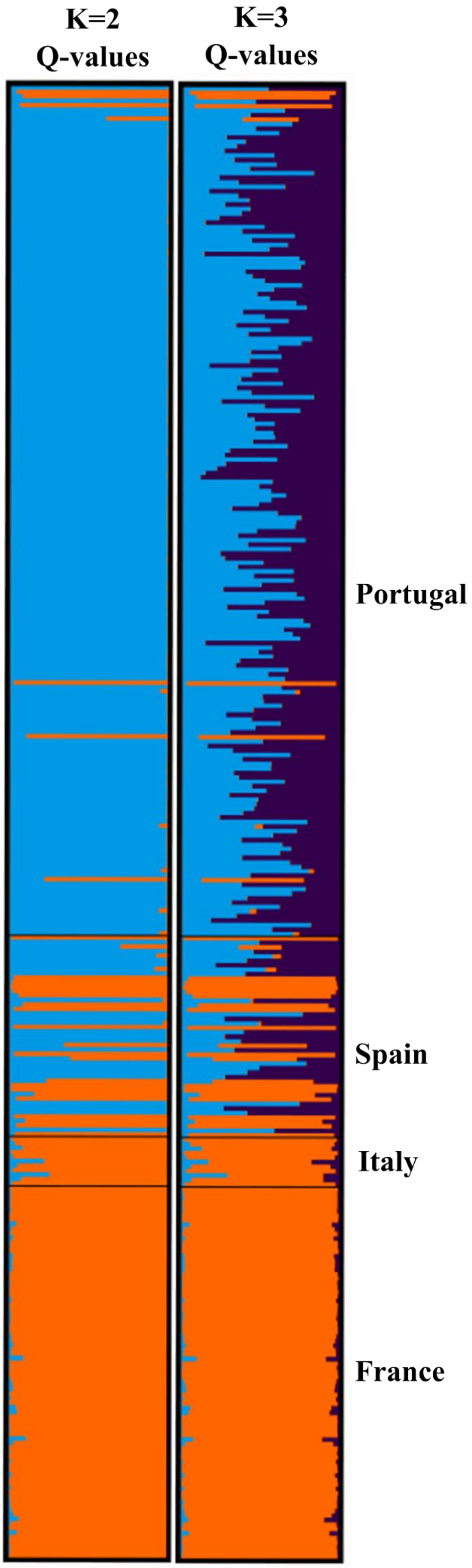

Figure 11: European dataset. STRUCTURE plot showing the membership partitioning into two (K = 2) and three (K = 3) clusters. Each individual is represented by a horizontal bar. Horizontal black lines separate individuals from different populations

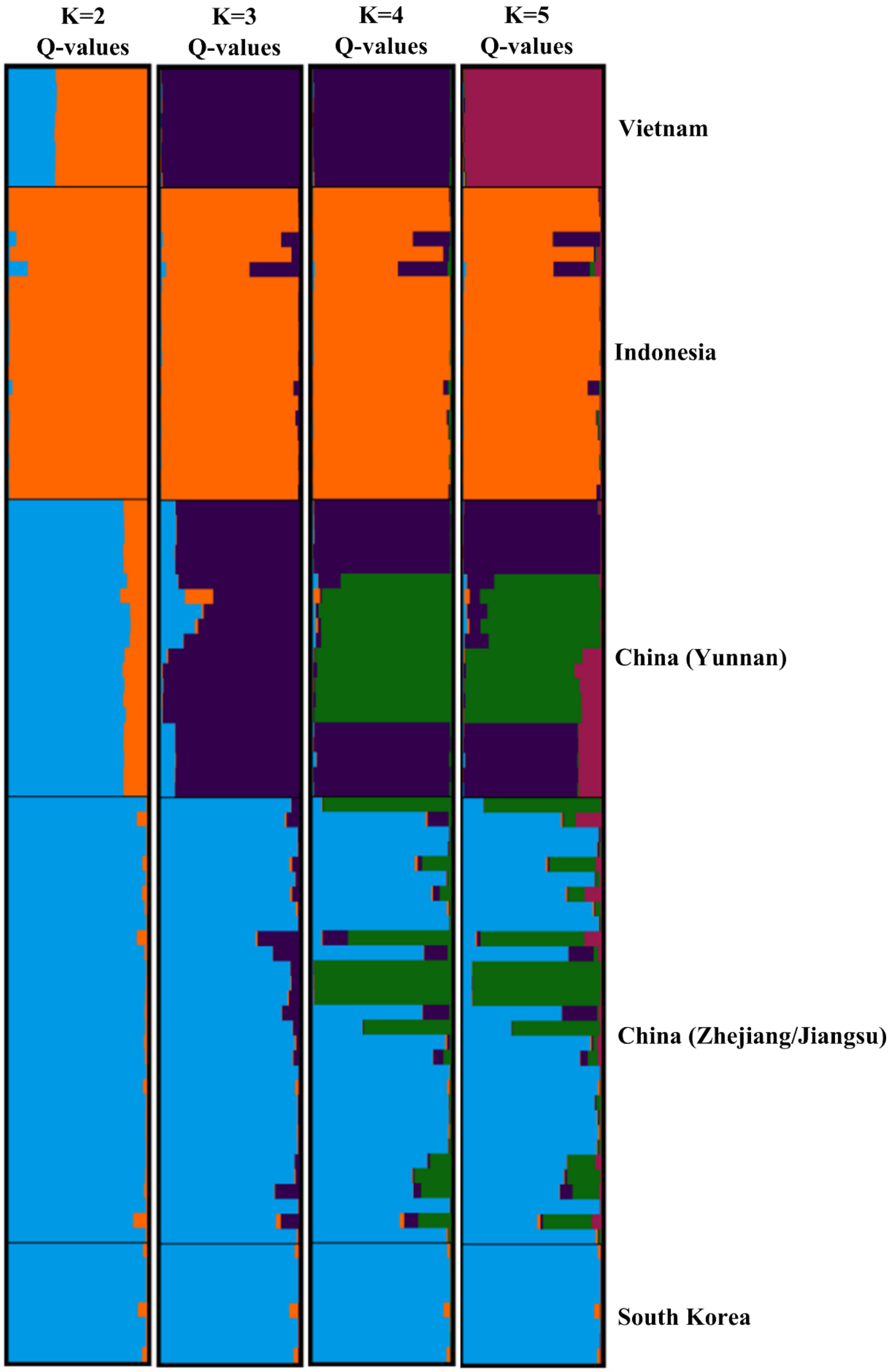

Figure 12: Asian dataset. STRUCTURE plot showing the membership partitioning into two (K = 2), three (K = 3), four (K = 4) and five (K = 5) clusters. Each individual is represented by a horizontal bar. Horizontal black lines separate individuals from different populations

Multi-locus F_{ST} values between populations of the invaded and native ranges are shown in Table 6. The least divergent populations in Europe were the Portuguese and Spanish (0.070), whereas the populations of Portugal and Italy showed the highest level of differentiation (0.325). Pairwise F_{ST} values between European and Asian populations produced the lowest differentiation from comparisons with the Chinese population of Zhejiang/Jiangsu, being the lowest F_{ST} obtained for France (0.113) and the highest for Portugal (0.366). The greatest divergence was observed for comparisons with the population of Vietnam, being again the lowest F_{ST} with France (0.341) and the highest with Portugal (0.561).

Table 6: Multi-locus estimates of F_{ST} (below diagonal) and P-values (above diagonal) between populations of the invaded native ranges

	Portugal	**Spain**	**Italy**	**France**	**South Korea**	**Vietnam**	**Indonesia**	**China Yunnan**	**China Zhejiang/ Jiangsu**
Portugal		**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**
Spain	0.070		**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**
Italy	0.325	0.193		**0.00000**	**0.00002**	**0.00000**	**0.00000**	**0.00000**	**0.00000**
France	0.249	0.116	0.138		**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**
South Korea	0.438	0.311	0.336	0.183		**0.00021**	**0.00000**	**0.00357**	**0.00000**
Vietnam	0.561	0.445	0.441	0.341	0.258		**0.00000**	**0.00000**	**0.00000**
Indonesia	0.495	0.373	0.344	0.259	0.245	0.302		**0.00000**	**0.00000**
China Yunnan	0.459	0.308	0.267	0.207	0.058	0.189	0.135		0.99894
China Zhejiang/ Jiangsu	0.366	0.232	0.218	0.113	0.070	0.159	0.174	-0.016	

P-values in bold are significant following sequential Bonferroni correction ($\alpha = 0.05$, $k = 36$)

d. Hardy-Weinberg Equilibrium

P-values for Hardy-Weinberg equilibrium (HWE), heterozygote deficiency, and heterozygote excess, and respective standard errors, per locus and population are shown in Table 7. Fisher's exact tests for HWE applied at individual loci yielded 33 significant P-values, following sequential Bonferroni correction, most of which were exhibited by populations of Indonesia (10), the Chinese province of Yunnan (9), and France (7). Within the study area, both Iberian populations had loci out of HWE (Spain: 4 loci; Portugal: 2 loci), whereas the Italian population did not show any departure from HWE. Disequilibrium was caused by a deficiency of heterozygotes for every single locus (P-value ≤ 0.0012, U-test for heterozygote deficiency). Locus R1-137 produced the greatest number of HWE deviations (4; P-value ≤ 0.0020, Fisher's exact test), whereas locus R1-75 did not show any HWE deviation.

Fisher's exact test across loci revealed highly significant departures from HWE for all native and invasive populations (P-value ≤ 0.0003), except for the Italian (P-value = 0.9032), South Korean (P-value = 0.2001), and Vietnamese (P-value = 0.4803). However, the multi-locus U-test for heterozygote deficiency did not fully support Fisher's exact test results producing a non-significant P-value for the population of Yunnam (0.0941) and a significant P-value for the population of Vietnam (0.0336), although this became non-significant after sequential Bonferroni correction (Table 7).

Table 7: Exact test probability of HWE, U-test probability of heterozygote deficiency and heterozygote excess, and respective standard errors for each locus and across loci, per population

		Portugal	**Spain**	**Italy**	**France**	**South Korea**	**Vietnam**	**Indonesia**	**China (Yunnan)**	**China (Zhejiang/ Jiangsu)**
R1-36	P (HWE)	0.8498	1.0000	0.4394	0.8501	0.0858	1.0000	**0.0035**	**0.0033**	0.0249
	± SE	±0.0020	±0.0000	±0.0019	±0.0043	±0.0026	±0.0000	±0.0015	±0.0011	±0.0033
	P (ht. def.)	0.4526	0.6957	0.4394	0.4015	0.5821	0.4343	**0.0001**	0.3247	0.0227
	± SE	±0.0061	±0.0045	±0.0019	±0.0137	±0.0047	±0.0051	±0.0001	±0.0167	±0.0031
	P (ht. exc.)	0.6902	0.5541	0.9612	0.7244	0.4432	0.8234	0.9999	0.6831	0.9773
	± SE	±0.0058	±0.0048	±0.0010	±0.0129	±0.0047	±0.0044	±0.0001	±0.0164	±0.0031
R1-169	P (HWE)	0.7593	0.3558	-	0.0327	1.0000	1.0000	**0.0041**	**0.0003**	0.3053
	± SE	±0.0034	±0.0033		±0.0016	±0.0000	±0.0000	±0.0008	±0.0003	±0.0128
	P (ht. def.)	0.7401	0.9305	-	0.0300	0.9118	1.0000	0.0266	0.4759	0.7683
	± SE	±0.0082	±0.0021		±0.0015	±0.0017	±0.0000	±0.0014	±0.0147	±0.0157
	P (ht. exc.)	0.3688	0.1964	-	0.9929	0.5602	0.9347	0.9752	0.5519	0.3046
	± SE	±0.0088	±0.0033		±0.0007	±0.0023	±0.0014	±0.0013	±0.0148	±0.016
R4-114	P (HWE)	0.2124	0.0131	0.5407	0.0954	1.0000	0.2870	**0.0000**	0.0074	0.0268
	± SE	±0.0045	±0.0015	±0.0019	±0.0067	±0.0000	±0.0067	±0.0000	±0.0016	±0.0054
	P (ht. def.)	0.1101	**0.0040**	0.3273	0.0358	0.8613	0.2155	**0.0000**	0.6548	0.4282
	± SE	±0.0043	±0.0006	±0.0026	±0.0033	±0.0074	±0.0065	±0.0000	±0.0122	±0.0204
	P (ht. exc.)	0.9378	0.9984	0.9571	0.9647	0.4144	0.8055	1.0000	0.3951	0.5820
	± SE	±0.0032	±0.0003	±0.0012	±0.0033	±0.0112	±0.006	±0.0000	±0.0125	±0.0199
D3-15	P (HWE)	**0.0000**	**0.0006**	1.0000	**0.0002**	0.5853	0.2726	1.0000	0.3088	0.4670
	± SE	±0.0000	±0.0002	±0.0000	±0.0001	±0.0109	±0.0040	±0.0000	±0.0092	±0.0234
	P (ht. def.)	**0.0000**	**0.0001**	1.0000	0.0201	0.5853	0.2460	1.0000	0.9256	0.0692
	± SE	±0.0000	±0.0000	±0.0000	±0.0034	±0.0109	±0.0035	±0.0000	±0.0058	±0.012
	P (ht. exc.)	1.0000	0.9999	0.5625	0.9799	0.7662	0.9047	0.9150	0.1264	0.9318
	± SE	±0.0000	±0.0000	±0.0020	±0.0034	±0.0075	±0.0028	±0.0039	±0.0068	±0.0120

Table 7: Continued

		Portugal	**Spain**	**Italy**	**France**	**South Korea**	**Vietnam**	**Indonesia**	**China (Yunnan)**	**China (Zhejiang/ Jiangsu)**
LIST2015	P (HWE)	0.0371	0.0108	1.0000	**0.0000**	0.1144	0.3841	**0.0007**	0.0360	0.7047
	± SE	±0.0046	±0.0018	±0.0000	±0.0000	±0.0036	±0.0055	±0.0005	±0.0066	±0.0210
	P (ht. def.)	0.0146	0.0871	0.5520	0.0116	0.0763	0.3841	**0.0000**	0.1869	0.9615
	± SE	±0.0024	±0.0059	±0.0025	±0.0038	±0.0033	±0.0055	±0.0000	±0.0139	±0.0080
	P (ht. exc.)	0.9864	0.9132	0.8646	0.9884	0.9889	0.7990	1.0000	0.8150	0.0611
	± SE	±0.0024	±0.0059	±0.0018	±0.0038	±0.0011	±0.0043	±0.0000	±0.0139	±0.0104
LIST2020B	P (HWE)	0.0172	0.0714	0.5352	**0.0067**	1.0000	0.5919	**0.0000**	**0.0000**	0.0984
	± SE	±0.0007	±0.0035	±0.0020	±0.0017	±0.0000	±0.0096	±0.0000	±0.0000	±0.0079
	P (ht. def.)	0.0172	0.0460	0.4021	0.3226	1.0000	0.5919	0.2431	0.0671	1.0000
	± SE	±0.0007	±0.0028	±0.0024	±0.0174	±0.0000	±0.0096	±0.0215	±0.0108	±0.0000
	P (ht. exc.)	1.0000	0.9952	0.9425	0.6976	0.4860	0.7784	0.8427	0.9336	0.0111
	± SE	±0.0000	±0.0008	±0.0013	±0.0173	±0.0017	±0.0067	±0.0169	±0.0108	±0.0016
R4-33	P (HWE)	0.7459	1.0000	0.2577	**0.0002**	0.5951	0.2812	1.0000	0.3105	0.4204
	± SE	±0.0033	±0.0000	±0.0022	±0.0002	±0.0098	±0.0051	±0.0000	±0.0099	±0.0271
	P (ht. def.)	0.7530	0.7383	0.9836	0.0138	0.5951	0.2509	1.0000	0.9259	0.0874
	± SE	±0.0075	±0.0030	±0.0007	±0.0024	±0.0098	±0.0047	±0.0000	±0.0056	±0.0134
	P (ht. exc.)	0.3618	0.5494	0.2142	0.9862	0.7790	0.9029	0.9184	0.1271	0.9139
	± SE	±0.0083	±0.0034	±0.0023	±0.0024	±0.0073	±0.0031	±0.0039	±0.0071	±0.0132
R1-77	P (HWE)	-	-	-	**0.0000**	0.3285	0.5316	**0.0040**	**0.0008**	0.3045
	± SE				±0.0000	±0.0048	±0.0016	±0.0017	±0.0004	±0.0105
	P (ht. def.)	-	-	-	**0.0000**	1.0000	0.4428	**0.0012**	0.7257	0.3772
	± SE				±0.0000	±0.0000	±0.0023	±0.0006	±0.0111	±0.0123
	P (ht. exc.)	-	-	-	1.0000	0.0889	0.9492	0.9989	0.3114	0.6395
	± SE				±0.0000	±0.0027	±0.0011	±0.0006	±0.0107	±0.0124

Table 7: Continued

		Portugal	**Spain**	**Italy**	**France**	**South Korea**	**Vietnam**	**Indonesia**	**China (Yunnan)**	**China (Zhejiang/ Jiangsu)**
LIST2018B	P (HWE)	0.9727	**0.0037**	1.0000	**0.0016**	-	0.1115	1.0000	0.0161	-
	± SE	±0.0014	±0.0005	±0.0000	±0.0006		±0.0025	±0.0000	±0.0018	
	P (ht. def.)	0.4033	0.0184	0.5771	**0.0000**	-	0.2452	0.6936	0.4602	-
	± SE	±0.0123	±0.0015	±0.0115	±0.0000		±0.0039	±0.0091	±0.0092	
	P (ht. exc.)	0.5985	0.9816	0.5592	1.0000	-	0.8146	0.5241	0.6428	-
	± SE	±0.0123	±0.0015	±0.0116	±0.0000		±0.0036	±0.0121	±0.0092	
R1-137	P (HWE)	**0.0000**	**0.0020**	0.4811	0.1764	0.0492	0.4134	**0.0000**	**0.0000**	0.4173
	± SE	±0.0000	±0.0004	±0.0017	±0.0050	±0.0034	±0.0153	±0.0000	±0.0000	±0.0242
	P (ht. def.)	**0.0000**	0.0460	1.0000	0.0187	0.4596	0.1901	0.0203	0.3493	0.2338
	± SE	±0.0000	±0.0022	±0.0000	±0.0020	±0.0069	±0.0128	±0.0032	±0.0250	±0.0209
	P (ht. exc.)	1.0000	0.9541	0.2494	0.9824	0.7944	0.8689	0.9797	0.6596	0.7724
	± SE	±0.0000	±0.0021	±0.0020	±0.0020	±0.0055	±0.0121	±0.0032	±0.0250	±0.0205
D2-185	P (HWE)	0.5473	0.0273	1.0000	0.4256	0.3050	0.2629	**0.0048**	**0.0026**	0.0299
	± SE	±0.0060	±0.0013	±0.0000	±0.0098	±0.0093	±0.0080	±0.0014	±0.0013	±0.0056
	P (ht. def.)	0.8080	0.0159	0.6021	0.8863	0.5762	0.3800	**0.0007**	0.9730	0.1490
	± SE	±0.0065	±0.0010	±0.0027	±0.0085	±0.0095	±0.0081	±0.0003	±0.0031	±0.0127
	P (ht. exc.)	0.2843	0.9974	0.8422	0.1794	0.5118	0.7357	0.9993	0.0736	0.8611
	± SE	±0.0078	±0.0004	±0.0021	±0.0108	±0.0095	±0.0060	±0.0003	±0.0059	±0.0123
VMA-8	P (HWE)	0.4628	0.5469	0.2816	0.0238	-	0.7466	0.0720	**0.0000**	-
	± SE	±0.0113	±0.0033	±0.0022	±0.0047		±0.0153	±0.0120	±0.0000	
	P (ht. def.)	0.2501	0.8398	0.2816	**0.0021**	-	1.0000	0.3709	0.8905	-
	± SE	±0.0167	±0.0034	±0.0022	±0.0010		±0.0000	±0.0241	±0.0097	
	P (ht. exc.)	0.7777	0.3575	0.9921	0.9979	-	0.0740	0.8320	0.2597	-
	± SE	±0.0161	±0.0047	±0.0005	±0.0010		±0.0089	±0.0193	±0.0153	

Table 7: Continued

		Portugal	**Spain**	**Italy**	**France**	**South Korea**	**Vietnam**	**Indonesia**	**China (Yunnan)**	**China (Zhejiang/ Jiangsu)**
R1-80	P (HWE)	-	0.0328	-	0.8076	0.0800	1.0000	**0.0000**	**0.0000**	**0.0038**
	± SE		±0.0015		±0.0018	±0.0035	±0.0000	±0.0000	±0.0000	±0.0020
	P (ht. def.)	-	0.0328	-	0.4757	0.0559	1.0000	**0.0005**	0.0536	0.0118
	± SE		±0.0015		±0.0056	±0.0024	±0.0000	±0.0003	±0.0137	±0.0057
	P (ht. exc.)	-	0.9965	-	0.7076	0.9504	0.3212	0.9995	0.9502	0.9882
	± SE		±0.0004		±0.0050	±0.0023	±0.0109	±0.0003	±0.0121	±0.0057
R1-75	P (HWE)	0.5714	0.3403	0.0417	0.0512	0.1027	0.1938	0.0176	0.4635	0.3412
	± SE	±0.0116	±0.0053	±0.0020	±0.0029	±0.0028	±0.0046	±0.0015	±0.0046	±0.0073
	P (ht. def.)	0.8704	0.2496	0.0132	0.5817	0.1027	0.1938	**0.0044**	0.1267	0.9995
	± SE	±0.0160	±0.0057	±0.0009	±0.0088	±0.0028	±0.0046	±0.0007	±0.0028	±0.0003
	P (ht. exc.)	0.1647	0.7856	0.9990	0.4214	0.9441	0.9351	0.9970	0.9058	0.0097
	± SE	±0.0176	±0.0051	±0.0003	±0.0088	±0.0019	±0.0027	±0.0005	±0.0027	±0.0016
R4-100	P (HWE)	0.0386	0.0856	1.0000	**0.0000**	0.9024	0.1381	**0.0017**	**0.0000**	0.0200
	± SE	±0.0025	±0.0021	±0.0000	±0.0000	±0.0031	±0.0033	±0.0011	±0.0000	±0.0063
	P (ht. def.)	0.0221	0.0856	0.8697	**0.0000**	0.5118	0.0372	0.0297	**0.0000**	0.0007
	± SE	±0.0022	±0.0021	±0.0016	±0.0000	±0.0058	±0.0015	±0.0030	±0.0000	±0.0007
	P (ht. exc.)	0.9900	0.9854	0.5898	1.0000	0.6593	0.9950	0.9720	1.0000	0.9993
	± SE	±0.0014	±0.0007	±0.0023	±0.0000	±0.0061	±0.0006	±0.0028	±0.0000	±0.0007
VMA-6	P (HWE)	0.0561	**0.0000**	1.0000	0.4691	-	0.2002	0.0944	0.5934	-
	± SE	±0.0012	±0.0000	±0.0000	±0.0066		±0.0022	±0.0031	±0.0049	
	P (ht. def.)	0.0561	**0.0000**	0.6062	0.3544	-	0.2002	0.0944	0.7042	-
	± SE	±0.0012	±0.0000	±0.0024	±0.0114		±0.0022	±0.0031	±0.0076	
	P (ht. exc.)	0.9992	1.0000	0.8382	0.6659	-	1.0000	1.0000	0.4323	-
	± SE	±0.0001	±0.0000	±0.0022	±0.0110		±0.0000	±0.0000	±0.0084	

Table 7: Continued

	Portugal	**Spain**	**Italy**	**France**	**South Korea**	**Vietnam**	**Indonesia**	**China (Yunnan)**	**China (Zhejiang/ Jiangsu)**
P (HWE)	**0.0000**	**0.0000**	0.9032	**0.0000**	0.2001	0.4803	**0.0000**	**0.0000**	**0.0003**
P (ht. def.)	**0.0000**	**0.0000**	0.0521	**0.0000**	0.3111	0.0336	**0.0000**	0.0941	**0.0077**
± SE	±0.0000	±0.0000	±0.0013	±0.0000	±0.006	±0.0025	±0.0000	±0.0078	±0.0032
P (ht. exc.)	1.0000	1.0000	0.9479	1.0000	0.6889	0.9664	1.0000	0.9059	0.9923
± SE	±0.0000	±0.0000	±0.0013	±0.0000	±0.006	±0.0025	±0.0000	±0.0078	±0.0032

P (HWE), exact test probability of HWE; P (ht. def.), U-test probability of heterozygote deficiency; P (ht. exc.), U-test probability of heterozygote excess; SE, standard error. P-values in bold are significant following sequential Bonferroni correction (α = 0.05; k = 9)

While the presence of null alleles cannot be ruled out, it is possible that HW disequilibrium detected across most populations is caused by population sub-structuring, so that mixing of individuals of distinct gene pools, which can be originated from geographically distant locations, would cause a deficiency of heterozygotes (Wahlund effect). It should be noted that individuals of most populations examined by Arca et al. (2015) were sampled across a wide geographical range, where genetic structure was apparent. Among the three population samples examined herein only the Italian was possibly drawn from a single, randomly mating population as it was comprised of geographically close individuals. To assess the hypothesis of a Wahlund effect, populations from Portugal and Spain where sub-divided by geography and sampling year (as age structure can create Wahlund-like effects) and tested for heterozygote deficiency (Table 8). The U-test yielded non-significant P-values for most sub-populations of Portugal (9 out of 10), whereas two out of the three sub-populations of Spain showed a deviation from panmixia. However, the three significant P-values became non-significant when individuals that clustered with the French population in STRUCTURE analysis (purple cluster in Figure 8) were removed from the HWE analyses. These results suggest the presence of a Wahlund effect.

Table 8: U-test probability of heterozygote deficiency for the sub-populations of Portugal and Spain

Population	Sampling Year	N	P-value±SE
Portugal			
Viana do Castelo	2016	23	**0.0016±0.0002**
Viana do Castelo[1]	2016	20	0.4660±0.0039
Viana do Castelo	2017	10	0.3265±0.0027
Braga	2016	30	0.1870±0.0039
Braga[2]	2017	1	-
Porto[2]	2016	4	-
Porto	2017	9	0.8635±0.0015
Vila Real	2016	13	0.9872±0.0005
Vila Real	2017	11	0.0107±0.0005
Viseu[2]	2016	3	-
Viseu	2017	22	0.0108±0.0007
Aveiro	2017	26	0.4275±0.0041
Coimbra	2017	8	0.9594±0.0008
Leiria	2017	5	0.3966±0.0026
Spain			
A Coruña	2016	18	**0.0004±0.0001**
A Coruña[1]	2016	7	0.1810±0.0019
Pontevedra	2016	16	**0.0007±0.0001**
Pontevedra[1]	2016	9	0.6906±0.0027
Ourense	2016	6	0.0551±0.0010

[1] Individuals assigned to the French cluster in the STRUCTURE analysis were removed; [2] P-values for N < 5 were not calculated; P-values in bold are significant following sequential Bonferroni correction ($\alpha = 0.05$, $k = 16$)

e. Bottleneck

The occurrence of a recent bottleneck in the invasive populations was assessed using the Wilcoxon signed-rank test, for IA, SM, and TP mutational models (Table 9). While a bottleneck was consistently detected in the Spanish and Italian populations, in the Portuguese, French, and South Korean populations statistical significance varied with the mutational model used and population sample tested. Only IAM was able to detect the presence of a bottleneck across all invasive populations, producing the lowest significance levels (0.0148 < P-values < 0.0001). This result was unexpected given that SMM is more suited than IAM for microsatellite markers (Piry et al. 1999). In the Portuguese population sample, it was only when individuals with membership proportions assigned to the French cluster, in STRUCTURE analysis, were excluded from the analysis, that a consistent bottleneck signature was captured by every mutational model (0.0031 < P-value < 0.0005).

Table 9: Bottleneck tested in the invasive European and South Korean populations using three mutation models and different settings of TPM

Mutational model	TPM settings	Portugal[1] (N=190)	Portugal 2016[2] (N=98)	Portugal[3] (N=183)	Portugal[4] (N=94)	Spain (N=45)	Italy (N=11)	France (N=83)	South Korea (N=8)
IAM		**0.0067**	**0.0148**	**0.0012**	**0.0005**	**0.0001**	**0.0004**	**0.0001**	**0.0034**
SMM		0.2316	0.1788	**0.0031**	**0.0005**	**0.0075**	0.0148	0.3910	0.0955
TPM	σ=12; SMM=5%	**0.0123**	**0.0176**	**0.0012**	**0.0005**	**0.0001**	**0.0006**	**0.0003**	**0.0133**
	σ=12; SMM=95%	0.2131	0.1629	**0.0023**	**0.0005**	**0.0062**	0.0123	0.2319	0.0955
	σ=12; SMM=88%	0.1788	0.1629	**0.0023**	**0.0005**	**0.0017**	**0.0027**	0.1156	0.0955
	σ=30; SMM=20%	**0.0148**	**0.0209**	**0.0012**	**0.0005**	**0.0001**	**0.0006**	**0.0002**	**0.0164**
	σ=12; SMM=80%	0.1338	0.1629	**0.0023**	**0.0005**	**0.0007**	**0.0017**	0.0467	0.0955
	σ=30; SMM=70%	0.0290	0.1083	**0.0012**	**0.0005**	**0.0005**	**0.0013**	**0.0046**	0.0287
	σ=30; SMM=90%	0.1788	0.1629	**0.0023**	**0.0005**	**0.0011**	**0.0027**	0.1057	0.0955

[1]Population sample comprising all individuals collected in Portugal; [2]Population sample comprising only individuals collected in 2016; [3]Population sample excluding individuals assigned to the French cluster in STRUCTURE; [4]Population sample comprising only individuals collected in 2016, but excluding individuals assigned to the French cluster in STRUCTURE. P-values in bold are significant following sequential Bonferroni correction (α = 0.05, k = 8)

V. Discussion

Invasive alien species (IAS) are widely recognized as a significant component of human-caused global environmental change and are a major threat to biodiversity (Frankham et al. 2010; Villemant et al. 2011), having also a severe impact on agriculture and natural resources (Mack et al. 2000; Leza et al. 2018). Population genetics has become important to investigate biological invasions by providing the theoretical framework for deciphering migration pathways and demographic mechanisms underlying the colonization process (Choi et al. 2013). The importance of knowing these migration pathways lies on the possibility of simplifying the design of strategies to prevent future invasions and to control present invading populations (Estoup and Guillemaud 2010). The main goal of this study was to analyse the population structure and the genetic patterns of the invasion of the Asian hornet, *V. v. nigrithorax*, in Portugal, Spain, and Italy using mitochondrial and microsatellites markers.

To unravel the demographic history of a population it is important to integrate mitochondrial and microsatellites data (Zhang and Hewitt 2003). The mitochondrial DNA (mtDNA) is a single locus which is passed intact from queen to offspring. Although the strictly maternal inheritance is often seen as a drawback, in studies of eusocial species, such as *V. v. nigrithorax*, it allows the inference of the relative dispersal ability of females, providing important clues about the number of queens that have invaded the new environment (Latta 2006). On the other hand, microsatellites are nuclear codominant markers that have a high mutation rate, and consequently a high degree of polymorphism, which make them useful to search for signatures of recent evolutionary processes (Pearse and Crandall 2004), such as ongoing biological invasions.

1. Quality control of microsatellites data

While the software GIMLET did not find evidence of genotyping errors, MICRO-CHECKER detected null alleles in six loci (D3-15, LIST2020B, R1-137, LIST2018B, D2-185, and VMA-6). Detection of null alleles was primarily due to heterozygote deficiency and, coincidently, four of those loci (D3-15, R1-137, LIST2018B, and VMA-6) were also out of HWE in two of the focal populations (Portugal and Spain). However, HW deviations were likely caused by a Wahlund effect (see further details below). It is possible that, due to the sampling bias, probably MICRO-CHECKER did not have enough power to discriminate between the Wahlund effect and null alleles. Additionally, the focal populations showed reduce allelic diversity which is known to affect the performance of MICRO-CHECKER (Leimbach-Maus et al. 2018).

While the genotypes generated herein for the focal populations were consistently reproduced from two independent amplifications, the high amount of missing data (over 55%) for LIST2018B, VMA-8 and VMA-6 in the populations of Indonesia, the Chinese province of Zhejiang/Jiangsu, and South Korea (reference dataset) surveyed by Arca et al. (2015), suggests the occurrence of point mutations in the primer binding sites resulting in null alleles. This will appear as missing data if the locus is homozygous or as apparent homozygous if a heterozygous locus with one amplified allele (De Meeûs 2017).

2. Sample size effect in population structure analysis

The samples from Portugal, Spain, and Italy were merged with the dataset of Arca et al. (2015) to understand the origin of the populations and migration patterns. However, the resulting merged dataset had uneven sample sizes per population, ranging from eight (South Korea and Vietnam) to 190 individuals (Portugal). To examine the effect of uneven sample size in membership partitioning, three subsets of 20 individuals selected randomly from populations with $N \geq 20$ were tested. The results showed that the clustering arrangements were affected by variation in sample size. While in “Total” dataset membership partitioning occurred earlier in Europe (Figure 8) than in Asia, in the datasets with even sample sizes the opposite was observed. Similar findings were reported by Kalinowski (2011), who used computer simulation to examine the accuracy of STRUCTURE in summarizing population structure, and by Rosenberg et al. (2005), who tested subsets constructed from a dataset of 1 048 individuals from the Human Genome Diversity Project-Centre d’Etude du Polymorphisme Humain (HGDP-CEPH) Human Genome Diversity Panel to conclude that the sample size has a direct relationship with grouping.

3. Wahlund and migrants effect in Hardy-Weinberg equilibrium

The analysis of HWE is central in the study of population genetics since any deviation from it can provide clues for the inference on the functioning of the targeted populations (Selkoe and Toonen 2006). When populations represented in the merged dataset were tested, only those from Italy, the Chinese province of Yunnan, and the South Korean conformed to HW expectations. Deviations from HWE at particular loci can be due to several technical or biological factors, operating separately or in conjunction, which range from genotyping errors (e.g. null alleles), random sampling error, assertive mating, selection, finite populations size, to age or population structure (reviewed by Waples 2014). In the invasive populations of Iberia, individuals were sampled from geographically distant locations within each country and in different years. This means that if there is a population and/or age structure, pooling individuals originating from different genetic clusters into a single sample will lead to a deficit of heterozygotes, causing a Wahlund effect. The hypothesis of a Wahlund effect was tested in the Iberian populations by examining HWE in sub-populations defined according to sampling region and sampling year and by removing migrants identified in the STRUCTURE analysis. Since all sub-populations were in HWE, the hypothesis of a Wahlund effect was confirmed on the Iberian populations and its occurrence can be due to: (i) population structure, since sampled individuals are from distant places within the countries and, thus are from different breeding units; (ii) the presence of migrants that introduced a new gene pool, which did not yet mate within the established population (Waples 2014; Pinto et al. 2005).

4. Migration patterns of *V. v. nigrithorax*

Arca et al. (2015) have shown that the entry in France by *V. v. nigrithorax* was achieved by a single female hornet fertilized by 3.6 haploid males, originated from the Chinese province of Zhejiang/Jiangsu. The results obtained with the COI sequences and the microsatellites in Iberia and Italy support this migration pattern. A single mitochondrial haplotype was found in the three focal populations (haplotype F), the same as in France and in the UK (Arca et al. 2015; Budge et al. 2017). Among the native populations, haplotype F was only found in the Chinese province of Zhejiang/Jiangsu. The mitochondrial data is congruent with microsatellites, which showed that, among the native populations, Zhejiang/Jiangsu had the lowest differentiation with the European populations. The nuclear and mitochondrial data suggested that the invasion of *V. v. nigrithorax* throughout Europe is a continued expansion from the French introduction.

There is only one mitochondrial haplotype in Europe and the diversity in Iberia and Italy is lower than that of France. Finally, most of the alleles are shared among the European populations.

The very first individual of *V. v. nigrithorax* in Europe was reported in France in 2004 (Villemant et al. 2011). At this moment, the Asian hornet is well established in Europe and it already spread to most of the neighbouring countries of France. From France, *V. v. nigrithorax* expanded to Spain, in 2010; initially in Navarre and, later on that year, in the Basque Country. According to Goldarazena et al. (2015), the Asian hornet dispersed from France via the northern valleys of the Pyrenees. In 2011, the first Asian hornet was spotted in the North of Portugal and, in the following year, 2012, it arrived in Galicia. The integration of the time reports together with the genetic data provide important clues about the spreading pathways.

Of the three populations under study, the Spanish holds the highest genetic diversity. The PCoA and STRUCTURE analysis clustered almost half of the individuals (19 out of 45) from this population between the Portuguese and the French clusters (Figure 8 and 11). Thus, suggesting two independent introductions in Spain: one, that occurred in 2010, when the Asian hornet naturally spread through the northern valleys of the Pyrenees, the second introduction, in 2012, via expansion from the Portuguese population. The clustering and diversity patterns (shared haplotypes and alleles) together with the low genetic diversity observed in the Portuguese population suggests the occurrence of a founder effect in Portugal originating from a French source population. Among the individuals sampled in Portugal, there were six out of 190, that clustered with the French population (Figure 8), of which three of them were collected in 2016 in the north of Portugal (Viana do Castelo) and the remaining three were collected in 2017 further south, in centre of Portugal (Aveiro, Viseu, and Castelo Branco). Given the high dispersal rate of the hornet, the six migrants could have arrived by local movements or, alternatively, by human-mediated jump, especially in the case of the individuals from the centre.

In 2012, two individuals of the Asian hornet were reported in Italy (Demichelis et al. 2014). The genetic patterns depicted in the PCoA and STRUCTURE charts, which showed the Italian individuals clustering together with the individuals from France, supports the assumption of Bertolino et al. (2016) of a natural spread from France through a narrow corridor along the North-western coast.

In summary, the mitochondrial and nuclear data showed that the spread of *V. v. nigrithorax* throughout Europe seemingly results from a combination of a natural dispersal with a diffusion-like process and sudden jumps due to the passive transportation of gynes mediated by humans (Monceau et al. 2014)

5. Bottleneck simulations

Invasive populations are characterized by suffering a founder effect, which results in a smaller effective population size than the source (Choi et al. 2013). The reduction in the effective population size is characterized by a decrease in both number of alleles and heterozygosity. However, the reduction of the allele diversity is faster, due to the rapid loss of rare alleles, which will only have a small effect on the heterozygosity (Cristescu et al. 2010). This results in an excess of the heterozygosity considering the small number of alleles. The invasive population from France corroborates this prediction showing a clear genetic diversity loss, with only 58% and 73% of the allelic diversity and observed heterozygosity, respectively, reported by Arca et al. (2015) for the native source population of China (Zhejiang/Jiangsu). Reduction in diversity was similarly severe in the focal populations relatively to the population of France from where they spread, showing 56%, 59%, and 50% of allelic diversity and 63%, 71%, and 77% of observed heterozygosity, for Portugal, Spain, and Italy, respectively. This severe reduction supports the hypothesis of the occurrence of a new bottleneck for these populations.

The hypothesis of a bottleneck was tested in the three focal populations by using a variety of mutational models that have been employed by others (Piry et al. 1999; Cristescu et al. 2010; Bounas et al. 2018; Sacks and Milburn 2018; Lalis et al. 2019; Horreo et al. 2019; Coster et al. 2019). While in the Spanish and Italian populations the bottleneck was detected by all mutational methods (IAM, SMM and TPM), in the Portuguese population bottleneck was only detected by IAM and TPM with a small proportion of SMM (σ=12, SMM=5% and σ=30, SMM=20%). The IAM model seems to be more sensitive to the genotype data from Portugal, even though it contradicts the mutational model (SMM) that is recommended for microsatellites by the authors of the BOTTLENECK software (Piry et al. 1999). However, the fact that the microsatellites used in this study were either dinucleotic or imperfect repeats can be an explanation for the suitability of IAM to detect a bottleneck, as supported by simulations done by Cornuet and Luikart (1996), Cristescu et al. (2010) and Le Page et al. (2000). But, the low allelic diversity is probably a better explanation, since the analysis were able to detected a bottleneck for the populations from Spain and Italy.

VI. Conclusion

This study suggests that the spread of *V. v. nigrithorax* from France to the neighbouring countries has been undertaken in two different ways. While *V. v. nigrithorax* entered Italy and northern Spain (via Basque Country) by local natural dispersion, the invasive process in Portugal started with founder gynes that entered through a long-distance human-mediated jump, just as recently happened in the UK (Budge et al. 2017). Since then, the foundress population has rapidly expanded northward (into Galicia) and southward and it is currently distributed along most of the Iberian Atlantic coast. Meanwhile, the south-westward expansion from the Basque Country is bringing in new diversity into Galicia and more recently into Portugal as revealed by the six migrants identified in this study (Figure 9). The migrants exhibited a membership proportion in the French cluster of over 0.7, suggesting that there has not been inter-crossing with the established populations of *V. v. nigrithorax.*

As in any founding event, there was a severe loss of diversity. However, this loss was not sufficient to prevent a successful colonization of large tracts of the Portuguese and Galician territories. The diet generalization and broad nesting site preference, as well as, the similar climatic conditions similar to those found in the native range, have facilitated the successful and fast establishment of the Asian hornet (Choi et al. 2013).

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Appendix

Table S1: List of samples genotyped in this study. Samples sequenced for COI are in bold.

Sample ID	Country	Coord. Y	Coord. X	Year	R1-36		R1-169		R4-114		D3-15		LIST 2015		LIST 2020B		R4-33		R1-77		LIST 2018B		R1-137		D2-185		VMA-8		R1-80		R1-75		R4-100		VMA-6	
1	Portugal	41.71500	-8.83389	2016	107	107	163	163	128	138	166	166	180	186	189	189	205	209	253	253	113	121	186	186	214	214	251	267	110	110	152	156	180	184	244	244
2	**Portugal**	**41.71281**	**-8.83588**	**2016**	**99**	**107**	**158**	**163**	**138**	**138**	**166**	**170**	**178**	**180**	**195**	**195**	**205**	**205**	**253**	**253**	**113**	**113**	**182**	**190**	**208**	**208**	**251**	**267**	**110**	**110**	**144**	**156**	**180**	**180**	**244**	**244**
3	Portugal	41.71303	-8.83588	2016	99	99	163	163	128	128	160	160	178	180	189	189	205	205	253	253	113	113	182	186	208	208	251	251	110	110	156	156	180	180	242	244
5	Portugal	41.69528	-8.74861	2016	99	107	163	163	128	138	166	166	180	186	189	189	205	205	253	253	121	137	186	186	208	208	251	267	110	110	152	156	180	180	244	244
9	Portugal	41.71500	-8.83389	2016	99	107	158	163	128	128	160	160	180	180	189	189	205	209	253	253	113	113	182	186	208	208	251	251	110	110	156	156	180	180	242	244
12	Portugal	41.68528	-8.74667	2016	99	107	158	163	138	138	166	166	180	180	189	189	205	209	253	253	113	137	186	186	208	214	267	267	110	110	152	156	180	180	244	244
15	Portugal	40.53631	-8.47097	2017	99	107	163	163	138	138	166	166	180	186	189	189	205	209	253	253	137	137	186	186	208	214	267	267	110	110	156	156	180	184	244	244
16	**Portugal**	**40.59890**	**-8.30717**	**2017**	**99**	**99**	**158**	**163**	**138**	**138**	**166**	**166**	**178**	**186**	**189**	**189**	**209**	**209**	**253**	**253**	**113**	**137**	**186**	**190**	**208**	**208**	**267**	**267**	**110**	**110**	**152**	**152**	**180**	**184**	**242**	**244**
63	**Portugal**	**41.48010**	**-7.79340**	**2016**	**99**	**99**	**163**	**163**	**128**	**138**	**166**	**166**	**180**	**186**	**189**	**189**	**209**	**209**	**253**	**253**	**121**	**137**	**186**	**186**	**208**	**214**	**251**	**267**	**110**	**110**	**152**	**152**	**180**	**180**	**244**	**244**
64	Portugal	41.52660	-7.80009	2016	99	107	158	163	128	138	166	166	186	186	189	189	205	205	253	253	113	137	186	186	208	214	251	267	110	110	152	156	180	184	244	244
65	Portugal	41.48391	-7.66751	2016	99	107	163	163	128	128	166	166	186	186	189	189	209	209	253	253	121	137	186	186	214	214	251	251	110	110	152	152	180	184	244	244
66	Portugal	41.53330	-7.60516	2016	99	107	158	163	128	138	166	166	180	186	189	189	205	209	253	253	121	121	186	186	208	214	251	267	110	110	156	156	180	184	244	244
67	Portugal	41.58979	-7.69481	2016	99	107	158	163	128	138	166	166	186	186	189	189	205	205	253	253	113	137	186	186	208	214	251	267	110	110	156	156	180	184	244	244
68	Portugal	41.53621	-7.78321	2016	99	99	158	163	128	128	166	166	180	186	189	189	205	209	253	253	113	137	186	186	208	214	251	267	110	110	156	156	180	184	244	244
69	Portugal	41.46824	-7.83154	2016	99	107	158	163	128	138	166	166	180	186	189	189	205	205	253	253	113	121	186	186	208	208	251	267	110	110	156	156	180	184	244	244
70	Portugal	41.60175	-8.33125	2016	99	107	163	163	128	138	166	166	180	186	189	189	205	205	253	253	113	113	186	186	208	214	267	267	110	110	152	156	180	180	244	244
71	Portugal	41.56597	-8.72903	2016	99	99	158	158	128	128	166	166	186	186	189	189	205	205	253	253	113	121	186	186	208	208	251	251	110	110	152	156	180	184	244	244
72	**Portugal**	**41.57413**	**-8.15476**	**2016**	**99**	**99**	**158**	**158**	**138**	**138**	**166**	**166**	**186**	**186**	**189**	**189**	**205**	**209**	**253**	**253**	**113**	**121**	**186**	**186**	**214**	**214**	**251**	**267**	**110**	**110**	**152**	**152**	**180**	**184**	**244**	**244**
73	Portugal	41.64728	-8.10169	2016	99	107	163	163	128	138	166	166	186	186	189	189	205	205	253	253	121	137	186	186	208	214	267	267	110	110	152	156	180	180	244	244
74	Portugal	41.74853	-8.20900	2016	99	107	158	163	138	138	166	166	186	186	189	189	205	209	253	253	113	137	186	186	214	214	251	251	110	110	152	156	180	180	244	244
75	Portugal	41.48227	-8.45314	2016	99	99	163	163	138	138	166	166	186	186	189	189	205	205	253	253	121	121	186	186	208	208	267	267	110	110	156	156	180	184	244	244
76	Portugal	41.49271	-8.41702	2016	99	99	158	163	128	138	166	166	186	186	189	189	205	205	253	253	113	113	186	186	208	208	267	267	110	110	152	156	180	184	244	244
77	Portugal	41.64034	-8.50162	2016	107	107	158	163	128	138	166	166	180	186	189	189	205	209	253	253	113	137	186	186	208	214	251	267	110	110	156	156	180	180	244	244
78	Portugal	41.58015	-8.17065	2016	99	99	163	163	128	128	166	166	180	186	189	189	205	205	253	253	137	137	186	186	214	214	267	267	110	110	156	156	184	184	244	244
79	Portugal	41.22724	-8.66064	2016	107	107	158	163	128	138	166	166	180	186	189	189	205	209	253	253	113	121	186	186	208	208	251	267	110	110	156	156	180	184	244	244

Table S1: Continued

Sample ID	Country	Coord. Y	Coord. X	Year	R1-36		R1-169		R4-114		D3-15		LIST 2015		LIST 2020B		R4-33		R1-77		LIST 2018B		R1-137		D2-185		VMA-8		R1-80		R1-75		R4-100		VMA-6	
80	Portugal	41.57589	-8.60231	2016	107	107	163	163	128	138	166	166	180	180	189	189	205	205	253	253	113	121	186	186	208	208	267	267	110	110	152	156	180	180	244	244
81	Portugal	41.61547	-8.42331	2016	99	107	158	163	138	138	166	166	180	180	189	189	205	205	253	253	113	137	186	186	208	214	267	267	110	110	152	156	184	184	244	244
82	Portugal	41.74853	-8.20900	2016	99	99	163	163	128	128	166	166	186	186	189	189	205	205	253	253	137	137	186	186	208	214	251	251	110	110	152	156	180	184	244	244
83	Portugal	41.39587	-8.08927	2016	99	99	158	158	128	138	166	166	186	186	189	189	205	205	253	253	121	137	186	186	208	214	251	251	110	110	152	156	180	184	244	244
84	Portugal	41.56719	-8.10673	2016	99	99	158	158	138	138	166	166	180	186	189	189	205	209	253	253	137	137	186	186	208	208	267	267	110	110	156	156	184	184	244	244
85	Portugal	41.56372	-8.73064	2016	99	99	158	163	128	138	166	166	180	180	189	189	205	209	253	253	113	121	186	186	208	214	267	269	110	110	156	156	184	184	244	244
86	Portugal	41.49027	-8.50213	2016	99	99	163	163	128	138	166	166	186	186	189	189	205	209	253	253	113	121	186	186	208	214	251	251	110	110	156	156	180	184	244	244
87	Portugal	41.45579	-8.52483	2016	99	99	163	163	128	128	166	166	186	186	189	189	205	209	253	253	113	121	186	186	214	214	251	267	110	110	156	156	180	184	244	244
88	Portugal	41.56597	-8.72903	2016	99	99	163	163	128	138	166	166	180	186	189	189	205	209	253	253	113	113	186	186	214	214	251	267	110	110	156	156	180	180	244	244
89	Portugal	41.45052	-8.41837	2016	99	99	163	163	138	138	166	166	186	186	189	189	205	209	253	253	121	137	186	186	214	214	267	267	110	110	152	156	180	184	244	244
90	Portugal	41.49048	-8.46645	2016	99	107	163	163	128	138	166	166	186	186	189	189	205	209	253	253	113	121	186	186	208	214	251	267	110	110	152	156	180	180	244	244
91	Portugal	41.37990	-8.62115	2016	99	99	158	158	128	138	166	166	180	186	189	189	205	209	253	253	121	137	186	186	214	214	251	267	110	110	152	156	180	180	244	244
92	Portugal	41.62119	-8.13683	2016	99	99	163	163	128	128	166	166	180	180	189	189	205	205	253	253	113	137	186	186	208	214	267	269	110	110	152	156	184	184	244	244
93	Portugal	41.40307	-8.48811	2016	99	107	163	163	138	138	166	166	186	186	189	189	205	209	253	253	113	121	186	186	208	214	251	267	110	110	152	156	180	180	244	244
94	Portugal	41.51784	-8.51145	2016	99	107	158	163	128	138	166	166	180	186	189	189	205	209	253	253	113	113	186	186	214	214	251	251	110	110	156	156	180	180	244	244
96	Portugal	41.40228	-8.08621	2016	107	107	158	163	138	138	166	166	180	186	189	189	209	209	253	253	113	113	186	186	214	214	251	267	110	110	152	156	180	184	244	244
99	Portugal	41.40448	-8.09863	2016	99	107	163	163	128	138	166	166	186	186	189	189	205	209	253	253	121	137	186	186	214	214	267	267	110	110	152	156	180	184	244	244
100	Portugal	41.42963	-8.08872	2016	99	99	158	163	128	138	166	166	180	186	189	189	205	205	253	253	113	121	186	186	214	214	267	267	110	110	152	156	180	180	244	244
102	Portugal	41.42511	-8.09882	2016	99	107	158	158	138	138	166	166	180	186	189	189	205	209	253	253	113	137	186	186	208	214	251	267	110	110	156	156	180	184	244	244
103	Portugal	41.94724	-8.24563	2016	99	107	163	163	138	138	166	166	186	186	189	189	205	205	253	253	113	113	186	186	208	214	251	267	110	110	152	156	180	180	244	244
104	Portugal	41.91098	-8.55434	2016	107	107	163	163	128	128	166	166	180	186	189	189	205	209	253	253	137	137	186	186	208	214	251	267	110	110	152	156	180	180	244	244
105	Portugal	41.78501	-8.85263	2016	99	107	158	163	138	138	166	166	180	186	189	189	205	205	253	253	113	121	186	186	208	208	267	267	110	110	152	156	180	180	244	244
106	Portugal	41.90568	-8.66392	2016	107	107	163	163	138	138	166	166	186	186	189	189	205	205	253	253	121	137	186	186	208	214	251	251	110	110	152	156	180	184	244	244
107	Portugal	41.81807	-8.25406	2016	99	99	163	163	138	138	166	166	186	186	189	189	205	205	253	253	113	113	186	186	208	214	251	267	110	110	152	156	180	180	244	244
108	Portugal	41.93104	-8.74761	2016	99	107	158	163	138	138	166	166	186	186	189	189	205	209	253	253	113	121	186	186	208	214	251	251	110	110	152	156	180	180	244	244
109	Portugal	41.75240	-8.78914	2016	99	99	158	163	138	138	166	166	186	186	189	189	205	205	253	253	113	121	186	186	214	214	267	267	110	110	152	156	180	184	244	244
110	**Portugal**	**42.09274**	**-8.29366**	**2016**	**99**	**107**	**163**	**163**	**138**	**138**	**166**	**166**	**186**	**186**	**189**	**189**	**205**	**209**	**253**	**253**	**121**	**121**	**186**	**186**	**208**	**214**	**251**	**251**	**110**	**110**	**152**	**156**	**180**	**184**	**244**	**244**
111	Portugal	41.88335	-8.20796	2016	99	99	158	163	128	138	166	166	180	186	189	189	205	205	253	253	113	137	186	186	214	214	251	267	110	110	152	152	180	180	244	244
112	Portugal	41.84525	-8.68194	2016	99	107	163	163	128	138	166	166	186	186	189	189	205	205	253	253	121	137	186	186	208	208	267	267	110	110	156	156	180	180	244	244
113	Portugal	41.81564	-8.81981	2016	99	107	158	163	138	138	166	166	186	186	189	189	205	205	253	253	121	137	186	186	214	214	251	251	110	110	152	156	180	184	244	244
114	Portugal	42.01316	-8.16817	2016	99	99	158	163	138	138	166	166	180	186	189	189	205	205	253	253	137	137	186	186	208	208	251	251	110	110	156	156	180	184	244	244
115	Portugal	41.80520	-8.84033	2016	99	99	163	163	138	138	166	166	186	186	189	189	205	209	253	253	137	137	186	186	214	214	251	251	110	110	152	152	180	180	244	244
116	Portugal	41.48771	-8.74116	2016	99	99	163	163	128	138	166	166	186	186	189	189	205	209	253	253	113	137	186	186	208	214	251	251	110	110	152	156	180	180	244	244

Table S1: Continued

Sample ID	Country	Coord. Y	Coord. X	Year	R1-36		R1-169		R4-114		D3-15		LIST 2015		LIST 2020B		R4-33		R1-77		LIST 2018B		R1-137		D2-185		VMA-8		R1-80		R1-75		R4-100		VMA-6	
117	**Portugal**	**41.82130**	**-8.39005**	**2016**	**99**	**99**	**163**	**163**	**128**	**128**	**166**	**166**	**186**	**186**	**189**	**189**	**205**	**209**	**253**	**253**	**121**	**137**	**186**	**186**	**208**	**208**	**251**	**251**	**110**	**110**	**152**	**152**	**180**	**180**	**244**	**244**
118	Portugal	41.73810	-8.78755	2016	99	107	158	158	128	128	166	166	180	186	189	189	205	205	253	253	137	137	186	186	208	214	251	267	110	110	152	156	180	180	244	244
119	Portugal	41.46140	-8.65256	2016	99	99	158	163	138	138	166	166	180	186	189	189	205	205	253	253	113	137	186	186	214	214	251	267	110	110	156	156	180	180	244	244
120	Portugal	41.68911	-8.61391	2016	99	99	158	163	128	138	166	166	180	186	189	189	205	209	253	253	113	137	186	186	208	214	251	267	110	110	156	156	180	184	244	244
126	Portugal	41.28739	-7.73899	2016	99	99	158	163	128	128	166	166	186	186	189	189	205	205	253	253	113	121	186	186	208	214	251	267	110	110	156	156	184	184	244	244
127	Portugal	41.28739	-7.73899	2016	99	99	163	163	128	138	166	166	180	186	189	189	209	209	253	253	121	137	186	186	208	214	267	267	110	110	152	152	180	180	244	244
128	Portugal	41.28062	-7.74013	2016	99	107	158	158	138	138	166	166	180	186	189	189	205	209	253	253	113	121	186	186	208	214	251	267	110	110	156	156	180	184	244	244
129	Portugal	41.52786	-7.80028	2016	99	99	158	163	138	138	166	166	186	186	189	189	205	205	253	253	121	137	186	186	214	214	251	267	110	110	156	156	180	184	244	244
130	Portugal	41.51709	-7.80396	2016	99	107	158	163	138	138	166	166	186	186	189	189	205	209	253	253	113	121	186	186	208	214	251	251	110	110	152	156	180	184	244	244
131	Portugal	41.28213	-8.47491	2016	99	99	163	163	128	138	166	166	180	186	189	189	205	209	253	253	121	121	186	186	208	214	267	267	110	110	156	156	180	180	244	244
132	Portugal	41.33185	-8.47159	2016	99	107	158	163	128	138	166	166	180	186	189	189	205	209	253	253	113	121	186	186	214	214	251	251	110	110	152	156	184	184	244	244
133	Portugal	40.96952	-8.53443	2016	99	107	158	163	138	138	166	166	180	186	189	189	205	209	253	253	113	113	186	186	214	214	251	251	110	110	152	156	180	180	244	244
134	Portugal	40.42073	-8.51989	2016	99	99	163	163	128	138	166	166	186	186	189	189	205	205	253	253	113	121	186	186	208	214	251	267	110	110	152	156	184	184	244	244
135	Portugal	41.66589	-8.47075	2016	99	99	163	163	138	138	166	166	180	186	189	189	205	205	253	253	113	113	186	186	208	208	251	267	110	110	152	156	180	184	244	244
136	Portugal	41.38771	-8.61293	2016	99	99	163	163	138	138	166	166	186	186	189	189	205	209	253	253	113	137	186	186	208	208	251	251	110	110	156	156	180	180	244	244
137	**Portugal**	**41.52406**	**-8.76086**	2016	99	99	158	158	138	138	166	166	186	186	189	189	205	205	253	253	121	121	186	186	208	208	251	267	110	110	152	156	180	180	244	244
138	Portugal	41.82450	-8.62767	2016	99	107	158	163	128	138	166	166	180	186	189	189	205	205	253	253	121	137	186	186	208	214	251	251	110	110	152	152	180	184	244	244
139	**Portugal**	**41.33502**	**-8.57648**	2016	99	107	158	163	138	138	166	166	186	186	189	189	205	205	253	253	113	113	186	186	208	214	267	267	110	110	156	156	180	180	244	244
140	Portugal	41.35036	-8.44174	2016	99	99	158	158	138	138	166	166	186	186	189	189	205	205	253	253	113	121	186	186	208	214	267	267	110	110	152	156	180	180	244	244
141	Portugal	41.32287	-8.63254	2016	99	107	158	163	128	138	166	166	186	186	189	189	205	209	253	253	121	137	186	186	208	214	251	267	110	110	152	156	180	180	244	244
142	Portugal	41.29414	-8.60549	2016	99	99	163	163	138	138	166	166	180	186	189	189	205	209	253	253	113	121	186	186	214	214	251	267	110	110	152	156	180	184	244	244
143	Portugal	41.46350	-8.55693	2016	99	99	158	163	128	138	166	166	186	186	189	189	205	209	253	253	113	121	186	186	208	214	251	267	110	110	152	156	180	184	244	244
144	Portugal	41.40844	-8.30906	2016	99	99	163	163	138	138	166	166	180	180	189	189	205	209	253	253	113	137	186	186	208	214	251	251	110	110	152	156	180	184	244	244
145	Portugal	41.40087	-8.24304	2016	99	99	158	163	128	138	166	166	186	186	189	189	205	205	253	253	113	113	186	186	208	208	251	267	110	110	152	156	184	184	244	244
146	Portugal	41.38799	-8.37584	2016	99	99	163	163	128	128	166	166	180	186	189	189	205	205	253	253	121	137	186	186	208	214	267	267	110	110	156	156	180	180	244	244
147	Portugal	41.32118	-8.72187	2016	99	99	163	163	138	138	166	166	186	186	189	189	205	209	253	253	113	121	186	186	214	214	251	267	110	110	152	156	180	184	244	244
148	Portugal	41.89902	-8.78400	2016	99	99	158	163	138	138	166	166	186	186	189	189	205	205	253	253	121	137	186	186	208	214	251	267	110	110	152	156	184	184	244	244
149	Portugal	41.93316	-8.59065	2016	99	107	158	163	128	128	166	166	186	186	189	189	205	209	253	253	121	121	186	186	208	214	267	267	110	110	152	156	180	184	244	244
150	Portugal	41.70732	-8.75599	2016	99	99	158	163	128	128	166	166	180	186	189	189	205	205	253	253	121	121	186	186	208	214	267	267	110	110	156	156	180	184	244	244
151	Portugal	-	-	2017	99	99	158	163	128	138	166	166	180	186	189	189	205	205	253	253	121	121	186	186	208	208	267	267	110	110	156	156	184	184	244	244
152	Portugal	40.48563	-8.52440	2017	99	99	158	163	128	128	166	166	186	186	189	189	205	209	253	253	113	121	186	186	214	214	251	267	110	110	152	156	180	180	244	244

Table S1: Continued

Sample ID	Country	Coord. Y	Coord. X	Year	R1-36		R1-169		R4-114		D3-15		LIST 2015		LIST 2020B		R4-33		R1-77		LIST 2018B		R1-137		D2-185		VMA-8		R1-80		R1-75		R4-100		VMA-6	
153	Portugal	40.60558	-7.99831	2016	107	107	158	163	128	138	166	166	180	186	189	189	205	205	253	253	121	137	186	186	214	214	251	267	110	110	156	156	180	180	244	244
154	Portugal	40.68076	-7.88644	2016	107	107	158	163	138	138	166	166	180	186	189	189	205	205	253	253	121	137	186	186	214	214	251	267	110	110	156	156	180	180	244	244
155	Portugal	40.64389	-8.30000	2017	99	99	163	163	128	138	166	166	186	186	189	189	205	209	253	253	113	137	186	186	214	214	267	267	110	110	152	156	180	180	244	244
156	**Portugal**	**41.69318**	**-7.67215**	**2016**	**99**	**107**	**158**	**163**	**138**	**138**	**166**	**166**	**180**	**186**	**189**	**189**	**205**	**209**	**253**	**253**	**113**	**121**	**186**	**186**	**208**	**214**	**251**	**251**	**110**	**110**	**156**	**156**	**180**	**184**	**244**	**244**
157	Portugal	41.69411	-7.66174	2016	99	99	158	163	138	138	166	166	180	186	189	189	205	209	253	253	113	121	186	186	214	214	251	267	110	110	156	156	180	184	244	244
158	Portugal	40.68076	-7.88644	2017	99	107	158	158	128	138	166	166	180	186	189	189	205	205	253	253	121	121	186	186	208	214	251	267	110	110	156	156	180	180	244	244
159	Portugal	41.46042	-7.68814	2017	99	99	158	163	138	138	166	166	180	180	189	189	205	209	253	253	113	121	186	186	208	214	267	267	110	110	152	152	180	180	244	244
160	Portugal	41.56898	-7.58456	2017	99	99	158	158	128	138	166	166	180	186	189	189	209	209	253	253	113	113	186	186	208	214	251	251	110	110	156	156	180	180	244	244
161	Portugal	41.50614	-7.65096	2017	99	99	158	163	138	138	166	166	186	186	189	189	209	209	253	253	137	137	186	186	208	214	251	267	110	110	156	156	180	180	244	244
162	Portugal	41.55747	-7.70981	2017	99	99	158	163	138	138	166	166	180	186	189	189	209	209	253	253	113	137	186	186	208	208	267	267	110	110	152	152	180	180	244	244
163	Portugal	41.59148	-7.63768	2017	99	99	163	163	128	128	166	166	186	186	189	189	205	205	253	253	121	121	186	186	214	214	251	251	110	110	152	156	180	180	244	244
164	Portugal	41.65650	-7.89117	2017	99	99	158	163	128	128	166	166	186	186	189	189	205	205	253	253	137	137	186	186	208	214	251	267	110	110	152	152	180	184	244	244
165	Portugal	40.51667	-8.08333	2017	99	99	158	158	138	138	166	166	186	186	189	189	205	205	253	253	113	137	186	186	214	214	251	251	110	110	152	152	180	180	244	244
166	Portugal	40.60972	-7.95500	2017	99	99	158	163	128	138	166	166	180	186	189	189	205	205	253	253	113	137	186	186	208	214	267	267	110	110	156	156	180	180	244	244
167	Portugal	40.48117	-8.44428	2017	99	99	158	163	138	138	166	166	186	186	189	189	205	205	253	253	113	113	186	186	214	214	251	251	110	110	152	156	180	184	244	244
168	Portugal	40.49140	-8.49790	2017	99	107	158	163	138	138	166	166	180	186	189	189	205	205	253	253	121	137	186	186	208	214	267	267	110	110	152	152	184	184	244	244
169	Portugal	40.39844	-8.63256	2017	99	99	158	163	128	138	166	166	180	186	189	189	205	209	253	253	113	137	186	186	208	208	267	267	110	110	152	152	184	184	244	244
170	Portugal	40.36354	-8.39606	2017	107	107	158	163	138	138	166	166	180	180	189	189	205	205	253	253	113	137	186	186	214	214	267	267	110	110	152	152	180	184	244	244
171	Portugal	40.62914	-8.44197	2017	99	99	163	163	138	138	166	166	180	186	189	189	205	205	253	253	113	121	186	186	208	214	251	267	110	110	152	152	184	184	244	244
172	Portugal	40.43499	-8.63643	2017	99	107	158	163	138	138	166	166	186	186	189	189	205	209	253	253	113	121	186	186	214	214	267	267	110	110	152	152	180	180	244	244
173	Portugal	41.42818	-8.50599	2017	99	99	163	163	128	138	166	166	180	180	189	189	205	209	253	253	121	137	186	186	214	214	251	251	110	110	156	156	180	184	244	244
174	Portugal	40.99894	-8.62103	2017	99	99	158	163	128	138	166	166	180	186	189	189	205	205	253	253	113	113	186	186	214	214	251	267	110	110	152	152	180	180	244	244
175	Portugal	40.34437	-8.26226	2017	99	107	158	158	138	138	166	166	180	186	189	189	205	205	253	253	137	137	186	186	208	214	267	267	110	110	152	156	180	180	244	244
176	Portugal	40.38027	-8.38478	2017	99	107	158	163	128	128	166	166	180	186	189	189	205	209	253	253	113	137	186	186	214	214	267	267	110	110	152	156	184	184	244	244
177	Portugal	40.43205	-8.15027	2017	99	107	158	163	138	138	166	166	186	186	189	189	205	209	253	253	113	137	186	186	208	214	251	267	110	110	156	156	180	180	244	244
178	Portugal	40.30204	-8.46103	2017	99	107	158	163	138	138	166	166	186	186	189	189	205	209	253	253	113	113	186	186	214	214	267	267	110	110	152	152	180	180	244	244
179	**Portugal**	**40.76955**	**-8.44757**	**2017**	**99**	**99**	**163**	**163**	**128**	**138**	**166**	**166**	**180**	**186**	**189**	**189**	**205**	**209**	**253**	**253**	**137**	**137**	**186**	**186**	**208**	**214**	**267**	**267**	**110**	**110**	**152**	**156**	**180**	**180**	**244**	**244**
180	Portugal	40.77848	-8.55547	2017	99	99	158	163	128	128	166	166	180	186	189	189	209	209	253	253	121	137	186	186	214	214	267	267	110	110	156	156	180	184	244	244
181	Portugal	40.52720	-8.28919	2017	99	99	158	163	138	138	166	166	186	186	189	189	205	205	253	253	113	121	186	186	208	214	251	267	110	110	156	156	180	180	244	244
182	Portugal	40.83911	-8.61918	2017	99	107	158	163	128	138	166	166	180	186	189	189	205	205	253	253	113	137	186	186	214	214	251	267	110	110	152	156	180	184	244	244
183	**Portugal**	**40.46490**	**-8.60276**	**2017**	**99**	**99**	**158**	**163**	**138**	**138**	**166**	**166**	**186**	**186**	**189**	**189**	**205**	**205**	**253**	**253**	**113**	**137**	**186**	**186**	**214**	**214**	**251**	**267**	**110**	**110**	**152**	**152**	**180**	**180**	**244**	**244**

Table S1: Continued

Sample ID	Country	Coord. Y	Coord. X	Year	R1-36		R1-169		R4-114		D3-15		LIST 2015		LIST 2020B		R4-33		R1-77		LIST 2018B		R1-137		D2-185		VMA-8		R1-80		R1-75		R4-100		VMA-6	
183	**Portugal**	**40.46490**	**-8.60276**	**2017**	**99**	**99**	**158**	**163**	**138**	**138**	**166**	**166**	**186**	**186**	**189**	**189**	**205**	**205**	**253**	**253**	**113**	**137**	**186**	**186**	**214**	**214**	**251**	**267**	**110**	**110**	**152**	**152**	**180**	**180**	**244**	**244**
184	Portugal	40.49207	-8.56860	2017	99	99	158	163	138	138	166	166	180	186	189	189	205	209	253	253	113	113	186	186	208	214	251	251	110	110	152	156	180	180	244	244
185	Portugal	40.48436	-8.52561	2017	99	99	163	163	128	138	166	166	180	186	189	189	209	209	253	253	137	137	186	186	214	214	267	267	110	110	152	156	180	180	244	244
186	Portugal	41.88859	-8.81480	2014	99	107	163	163	138	138	166	166	186	186	189	189	205	209	253	253	121	137	186	186	208	214	251	251	110	110	156	156	180	180	244	244
188	Portugal	41.88024	-8.81449	2017	99	99	163	163	138	138	166	166	186	186	189	189	205	205	253	253	113	137	186	186	208	214	251	267	110	110	152	152	180	180	244	244
206	Portugal	41.91729	-8.64726	2017	99	99	158	163	128	138	166	166	186	186	189	189	205	205	253	253	121	121	186	186	208	214	267	267	110	110	152	156	184	184	244	244
207	Portugal	41.87286	-8.69294	2017	99	99	163	163	128	138	166	166	186	186	189	189	205	205	253	253	113	137	186	186	208	208	251	251	110	110	152	152	180	184	244	244
208	Portugal	41.90792	-8.73847	2017	99	99	158	163	128	138	166	166	186	186	189	189	205	205	253	253	137	137	186	186	208	214	251	267	110	110	156	156	180	180	244	244
212	Portugal	41.91924	-8.76611	2017	99	99	158	163	128	138	166	166	186	186	189	189	205	209	253	253	113	113	186	186	208	214	251	267	110	110	152	156	180	184	244	244
213	Portugal	41.84078	-8.82205	2017	99	107	158	163	138	138	166	166	180	186	189	189	205	205	253	253	113	113	186	186	208	214	251	267	110	110	156	156	184	184	244	244
215	Portugal	41.85423	-8.81095	2017	99	107	158	158	138	138	166	166	180	186	189	189	205	209	253	253	121	137	186	186	208	214	267	267	110	110	152	156	180	184	244	244
240	Portugal	41.88955	-8.78842	2017	99	99	158	163	128	138	166	166	180	186	189	189	209	209	253	253	113	137	186	186	208	214	251	267	110	110	156	156	180	180	244	244
241	Portugal	41.97794	-8.69214	2017	107	107	163	163	128	138	166	166	186	186	189	189	205	205	253	253	121	137	186	186	208	214	251	267	110	110	152	156	180	180	244	244
243	Portugal	40.52703	-8.42985	2017	99	99	158	163	138	138	166	166	180	180	189	189	205	209	253	253	113	137	186	186	208	214	251	267	110	110	156	156	180	180	244	244
244	**Portugal**	**40.38840**	**-8.24360**	**2017**	**99**	**99**	**163**	**163**	**128**	**138**	**166**	**170**	**178**	**178**	**189**	**195**	**205**	**205**	**253**	**253**	**113**	**113**	**182**	**190**	**208**	**208**	**251**	**267**	**110**	**110**	**156**	**156**	**180**	**180**	**242**	**242**
245	Portugal	40.41496	-8.32067	2017	99	99	158	163	138	138	166	166	186	186	189	189	205	209	253	253	113	137	186	186	214	214	251	267	110	110	152	156	180	184	244	244
246	Portugal	40.38840	-8.24360	2017	99	107	158	163	128	138	166	166	186	192	189	189	205	209	253	253	113	121	186	186	208	214	251	251	110	110	152	152	180	180	244	244
247	Portugal	40.38840	-8.24360	2017	99	99	158	158	128	138	166	166	186	186	189	189	205	209	253	253	137	137	186	186	208	214	267	267	110	110	152	156	180	180	244	244
248	Portugal	40.41482	-8.20833	2017	99	107	158	163	128	138	166	166	180	186	189	189	205	205	253	253	113	137	186	186	214	214	251	267	110	110	152	152	180	184	244	244
249	Portugal	40.39946	-8.25153	2017	99	107	158	158	138	138	166	166	180	186	189	189	205	205	253	253	113	137	186	186	214	214	267	267	110	110	152	152	180	180	244	244
250	Portugal	40.66784	-7.91923	2017	99	99	158	163	138	138	166	166	180	186	189	189	205	209	253	253	113	121	186	186	208	214	251	267	110	110	156	156	180	184	244	244
253	Portugal	41.38111	-8.75667	2017	99	99	158	163	138	138	166	166	180	186	189	189	209	209	253	253	121	121	186	186	208	214	251	267	110	110	156	156	184	184	244	244
254	Portugal	40.62110	-8.56703	2017	99	107	158	158	128	138	166	166	186	186	189	189	205	209	253	253	113	137	186	186	214	214	267	267	110	110	152	156	180	184	244	244
255	Portugal	-	-	2014	99	99	163	163	138	138	166	166	186	186	189	189	205	205	253	253	113	113	186	186	208	208	0	0	110	110	156	156	180	180	244	244
256	Portugal	-	-	2014	99	107	163	163	128	138	166	166	180	186	189	189	205	205	253	253	121	137	186	186	214	214	251	251	110	110	152	156	180	184	244	244
257	Portugal	-	-	2014	99	99	158	163	128	138	166	166	180	186	189	189	205	209	253	253	121	121	186	186	208	214	251	267	110	110	152	156	180	184	244	244
258	**Portugal**	**40.51584**	**-7.96150**	**2017**	**107**	**107**	**158**	**158**	**138**	**138**	**166**	**166**	**180**	**180**	**189**	**195**	**205**	**205**	**253**	**253**	**113**	**113**	**182**	**182**	**208**	**208**	**251**	**267**	**110**	**110**	**152**	**156**	**180**	**184**	**242**	**244**
259	Portugal	40.67048	-7.95770	2017	99	107	158	158	128	138	166	166	186	186	189	189	205	205	253	253	113	121	186	186	208	214	251	267	110	110	152	156	180	180	244	244
260	Portugal	40.53610	-8.02387	2017	99	99	158	163	128	138	166	166	186	186	189	189	205	205	253	253	121	137	186	186	208	214	267	267	110	110	152	156	184	184	244	244
261	Portugal	40.66529	-7.95631	2017	99	99	158	163	138	138	166	166	186	186	189	189	205	205	253	253	137	137	186	186	208	214	251	267	110	110	152	156	184	184	244	244
262	Portugal	41.15162	-8.67534	2017	107	107	158	163	128	138	166	166	186	186	189	189	205	209	253	253	113	137	186	186	214	214	251	251	110	110	152	156	180	184	244	244
263	Portugal	41.15022	-8.61479	2017	99	107	163	163	128	128	166	166	180	186	189	189	205	209	253	253	113	121	186	186	208	214	251	267	110	110	152	156	180	184	244	244
264	Portugal	41.14271	-8.60423	2016	99	107	163	163	128	138	166	166	186	186	189	189	209	209	253	253	121	137	186	186	208	208	251	251	110	110	152	156	180	184	244	244

Table S1: Continued

Sample ID	Country	Coord. Y	Coord. X	Year	R1-36		R1-169		R4-114		D3-15		LIST 2015		LIST 2020B		R4-33		R1-77		LIST 2018B		R1-137		D2-185		VMA-8		R1-80		R1-75		R4-100		VMA-6	
265	Portugal	41.17924	-8.68692	2017	99	99	158	163	128	128	166	166	180	186	189	189	205	209	253	253	113	121	186	186	208	214	251	267	110	110	152	152	180	180	244	244
266	Portugal	41.15003	-8.61678	2017	99	99	158	163	128	128	166	166	186	186	189	189	205	209	253	253	137	137	186	186	208	214	251	267	110	110	156	156	180	184	244	244
267	**Portugal**	**41.14726**	**-8.58156**	**2017**	**99**	**107**	**158**	**158**	**128**	**138**	**166**	**166**	**186**	**186**	**189**	**189**	**205**	**209**	**253**	**253**	**137**	**137**	**186**	**186**	**214**	**214**	**251**	**267**	**110**	**110**	**152**	**156**	**184**	**184**	**244**	**244**
268	Portugal	41.15396	-8.64108	2016	99	107	158	158	128	138	166	166	186	186	189	189	209	209	253	253	113	137	186	186	214	214	251	267	110	110	152	156	180	184	244	244
269	Portugal	41.14596	-8.62091	2016	99	107	158	163	128	138	166	166	180	186	189	189	209	209	253	253	113	113	186	186	214	214	267	267	110	110	152	156	184	184	244	244
270	Portugal	41.18062	-8.63655	2017	99	99	163	163	138	138	166	166	186	186	189	189	205	209	253	253	113	137	186	186	208	214	267	267	110	110	152	156	180	184	244	244
272	Portugal	41.14143	-8.58983	2017	99	99	158	163	128	138	166	166	186	186	189	189	205	205	253	253	121	137	186	186	208	214	251	267	110	110	152	156	180	184	244	244
273	Portugal	41.17249	-8.58755	2017	99	99	163	163	138	138	166	166	186	186	189	189	205	209	253	253	113	137	186	186	208	214	267	267	110	110	156	156	180	184	244	244
275	**Portugal**	**40.91443**	**-7.96757**	**2017**	**99**	**99**	**163**	**163**	**128**	**138**	**166**	**166**	**186**	**186**	**189**	**189**	**209**	**209**	**253**	**253**	**121**	**137**	**186**	**186**	**208**	**208**	**251**	**267**	**110**	**110**	**152**	**156**	**180**	**184**	**244**	**244**
276	Portugal	40.95667	-7.78986	2017	99	107	158	158	128	138	166	166	186	186	189	189	205	205	253	253	113	121	186	186	208	214	251	267	110	110	156	156	180	180	244	244
278	Portugal	40.58349	-8.48257	2017	99	107	158	163	138	138	166	166	186	186	189	189	205	209	253	253	121	137	186	186	214	214	251	267	110	110	152	156	184	184	244	244
279	Portugal	40.60363	-8.45724	2017	99	107	158	158	128	128	166	166	180	186	189	189	205	205	253	253	113	137	186	186	208	214	267	267	110	110	152	156	180	184	244	244
283	Portugal	40.62479	-8.64224	2017	99	107	163	163	138	138	166	166	186	186	189	189	205	209	253	253	137	137	186	186	208	214	251	251	110	110	152	156	180	180	244	244
284	**Portugal**	**40.47051**	**-7.89951**	**2017**	**99**	**107**	**158**	**158**	**128**	**138**	**166**	**170**	**186**	**186**	**189**	**189**	**205**	**209**	**253**	**253**	**137**	**137**	**186**	**186**	**214**	**214**	**251**	**267**	**110**	**110**	**152**	**156**	**184**	**184**	**244**	**244**
285	**Portugal**	**40.32233**	**-8.02389**	**2017**	**99**	**99**	**158**	**163**	**138**	**138**	**166**	**166**	**180**	**186**	**189**	**189**	**205**	**209**	**253**	**253**	**113**	**137**	**186**	**186**	**208**	**214**	**251**	**267**	**110**	**110**	**152**	**156**	**180**	**180**	**244**	**244**
286	Portugal	40.21846	-8.13600	2017	99	107	158	163	128	138	166	166	186	186	189	189	209	209	253	253	113	113	186	186	208	214	251	267	110	110	152	152	184	184	244	244
287	Portugal	40.28409	-8.12017	2017	99	99	158	163	138	138	166	166	186	186	189	189	205	205	253	253	113	137	186	186	208	208	267	267	110	110	152	156	180	184	244	244
288	Portugal	40.65418	-8.22235	2017	99	99	158	158	128	138	166	166	186	186	189	189	205	209	253	253	113	137	186	186	214	214	251	267	110	110	156	156	180	180	244	244
289	Portugal	40.71552	-8.04064	2017	99	107	163	163	128	138	166	166	180	186	189	189	205	209	253	253	113	137	186	186	208	214	251	267	110	110	152	156	180	180	244	244
291	Portugal	41.31538	-7.75362	2017	99	99	163	163	128	138	166	166	186	186	189	189	205	209	253	253	113	137	186	186	208	214	267	267	110	110	152	156	180	180	244	244
292	Portugal	41.29086	-7.81527	2017	99	107	163	163	128	128	166	166	180	186	189	189	205	209	253	253	113	121	186	186	208	208	267	267	110	110	156	156	180	180	244	244
293	**Portugal**	**41.31694**	**-7.77111**	**2017**	**99**	**99**	**163**	**163**	**128**	**138**	**166**	**166**	**180**	**180**	**189**	**189**	**205**	**209**	**253**	**253**	**113**	**137**	**186**	**186**	**214**	**214**	**251**	**267**	**110**	**110**	**152**	**156**	**180**	**180**	**244**	**244**
294	Portugal	41.1599	-7.88990	2017	99	99	163	163	128	138	166	166	180	180	189	189	209	209	253	253	113	113	186	186	208	208	251	251	110	110	152	156	180	180	244	244
295	Portugal	39.7970	-8.29607	2017	99	107	163	163	128	138	166	170	186	186	189	189	209	209	253	253	113	113	186	186	214	214	251	267	110	110	152	156	180	180	244	244
308	Portugal	41.68414	-7.689480	2017	99	99	163	163	128	128	166	166	180	186	189	189	205	205	253	253	113	137	186	186	208	214	267	267	110	110	152	156	180	180	244	244
309	**Portugal**	**40.05764**	**-7.71991**	**2017**	**99**	**99**	**158**	**163**	**128**	**128**	**166**	**166**	**180**	**180**	**189**	**189**	**205**	**205**	**253**	**253**	**137**	**137**	**190**	**190**	**208**	**214**	**251**	**251**	**110**	**110**	**144**	**152**	**184**	**184**	**242**	**244**
310	Portugal	39.89271	-8.35188	2017	99	107	163	163	138	138	166	166	186	186	189	189	205	209	253	253	121	137	186	186	214	214	267	267	110	110	152	152	184	184	244	244
311	Portugal	39.97039	-8.46729	2017	99	107	158	163	138	138	166	166	186	186	189	189	205	205	253	253	113	113	186	186	208	214	251	251	110	110	152	156	180	180	244	244
312	Portugal	40.86056	-8.36626	2017	99	99	163	163	138	138	166	166	180	186	189	189	205	209	253	253	113	121	186	186	214	214	251	251	110	110	152	152	180	184	244	244
313	Portugal	40.75187	-8.31338	2017	99	99	158	163	128	128	166	166	180	186	189	189	205	209	253	253	113	121	186	186	208	214	267	267	110	110	152	156	180	180	244	244
314	Portugal	39.89513	-8.38223	2017	99	107	158	163	138	138	166	166	186	186	189	189	205	209	253	253	121	137	186	186	214	214	267	267	110	110	152	156	184	184	244	244
315	Portugal	39.88994	-8.36942	2017	99	99	158	158	138	138	166	166	180	186	189	189	205	209	253	253	113	113	186	186	214	214	251	267	110	110	152	156	180	184	244	244
316	Portugal	39.67831	-7.77562	2017	99	107	163	163	138	138	166	166	186	186	189	189	205	209	253	253	121	121	186	190	214	214	251	267	110	110	156	156	180	184	244	244

Table S1: Continued

Sample ID	Country	Coord. Y	Coord. X	Year	R1-36		R1-169		R4-114		D3-15		LIST 2015		LIST 2020B		R4-33		R1-77		LIST 2018B		R1-137		D2-185		VMA-8		R1-80		R1-75		R4-100		VMA-6	
317	Portugal	40.31010	-8.58957	2017	99	107	158	163	138	138	166	166	180	186	189	189	205	205	253	253	113	121	186	186	208	214	267	267	110	110	152	156	180	184	244	244
321	Portugal	40.38472	-7.95517	2017	99	99	158	163	128	138	166	166	180	186	189	189	205	205	253	253	113	121	186	186	208	214	251	267	110	110	156	156	184	184	244	244
322	**Portugal**	**41.19513**	**-7.01811**	**2017**	**99**	**99**	**158**	**163**	**138**	**138**	**166**	**166**	**186**	**186**	**189**	**189**	**209**	**209**	**253**	**253**	**113**	**113**	**186**	**186**	**208**	**214**	**251**	**267**	**110**	**110**	**152**	**156**	**180**	**180**	**244**	**244**
323	Portugal	40.51667	-7,85	2017	99	99	158	158	138	138	166	166	186	186	189	189	205	209	253	253	113	121	186	186	208	214	267	267	110	110	152	156	180	180	244	244
324	**Portugal**	**40.16139**	**-8.19977**	**2017**	**99**	**99**	**163**	**163**	**128**	**138**	**166**	**170**	**180**	**186**	**189**	**189**	**205**	**209**	**253**	**253**	**113**	**137**	**186**	**186**	**208**	**214**	**251**	**267**	**110**	**110**	**152**	**156**	**180**	**180**	**244**	**244**
18	**Espanha**	**42.53744**	**-8.62538**	**2016**	**99**	**107**	**163**	**163**	**128**	**128**	**170**	**170**	**178**	**180**	**195**	**195**	**205**	**209**	**253**	**253**	**113**	**113**	**182**	**190**	**208**	**208**	**251**	**251**	**108**	**108**	**156**	**156**	**180**	**184**	**244**	**244**
19	Espanha	43.68010	-7.89367	2016	99	99	158	163	138	138	166	166	186	186	189	189	205	209	253	253	113	113	186	186	208	214	267	267	110	110	156	156	180	184	244	244
20	Espanha	43.54263	-8.14058	2016	99	107	158	163	138	138	166	166	180	186	189	189	205	205	253	253	113	137	186	190	208	208	267	267	108	110	156	156	180	180	244	244
21	Espanha	43.69578	-7.94039	2016	99	99	158	163	128	138	166	166	180	186	189	189	205	205	253	253	137	137	186	186	208	214	251	267	110	110	156	156	180	180	244	244
22	Espanha	43.50278	-8.31978	2016	99	107	163	163	128	138	166	166	186	186	189	189	205	209	253	253	121	137	186	186	214	214	251	267	110	110	156	156	180	180	242	244
23	Espanha	43.44921	-8.22950	2016	99	107	158	163	128	138	166	166	180	180	189	189	205	205	253	253	113	113	186	186	214	214	267	267	110	110	152	156	180	184	244	244
24	Espanha	43.50387	-8.15904	2016	99	99	158	163	138	138	166	166	186	186	189	189	205	205	253	253	121	137	186	186	208	208	267	267	110	110	152	152	184	184	244	244
25	Espanha	43.52195	-8.03721	2016	99	99	158	163	128	138	166	166	186	186	189	189	205	209	253	253	121	137	186	186	208	214	251	267	110	110	144	156	180	180	244	244
26	Espanha	43.53605	-7.92434	2016	107	107	163	163	128	138	166	166	180	186	189	189	205	205	253	253	121	121	186	186	208	208	251	267	110	110	152	156	180	180	244	244
27	**Espanha**	**43.28755**	**-8.06458**	**2016**	**107**	**107**	**163**	**163**	**128**	**128**	**160**	**166**	**178**	**178**	**189**	**189**	**205**	**209**	**253**	**253**	**113**	**113**	**182**	**182**	**208**	**208**	**251**	**251**	**110**	**110**	**152**	**156**	**180**	**180**	**242**	**242**
29	Espanha	43.28895	-8.80320	2016	99	99	158	163	138	138	170	170	178	180	189	189	205	205	253	253	113	113	182	182	208	208	267	267	108	108	156	156	180	180	242	244
31	Espanha	42.87715	-8.55472	2016	99	107	158	163	128	138	166	170	180	180	195	195	205	205	253	253	113	113	182	190	208	208	267	267	108	108	156	156	180	180	242	242
32	Espanha	42.92957	-8.16061	2016	99	107	158	158	138	138	166	170	178	180	189	189	205	205	253	253	113	137	182	182	208	208	251	267	108	110	156	156	180	180	242	242
34	Espanha	42.41156	-8.76312	2016	99	107	158	163	128	128	160	160	180	180	189	189	205	209	253	253	113	113	182	186	208	208	251	251	110	110	156	156	180	180	242	244
35	Espanha	42.42115	-8.37457	2016	107	107	158	163	128	138	166	166	180	186	183	189	205	205	253	253	137	137	186	186	208	214	251	267	110	110	152	156	180	184	244	244
37	Espanha	42.26496	-8.84509	2016	99	107	158	163	128	128	166	166	178	178	189	189	205	209	253	253	113	113	182	186	208	208	251	267	110	110	152	156	180	180	242	242
39	**Espanha**	**42.20359**	**-8.70773**	**2016**	**99**	**107**	**163**	**163**	**128**	**128**	**160**	**160**	**178**	**180**	**189**	**189**	**205**	**209**	**253**	**253**	**113**	**113**	**182**	**186**	**208**	**208**	**251**	**267**	**110**	**110**	**156**	**156**	**180**	**180**	**242**	**242**
40	Espanha	42.17591	-8.63382	2016	99	107	158	163	138	138	166	166	186	186	189	189	205	209	253	253	113	137	186	186	208	214	251	267	110	110	152	156	180	180	244	244
41	Espanha	42.10775	-8.77865	2016	99	99	158	163	128	138	166	166	186	186	189	189	205	205	253	253	121	137	186	186	208	214	251	267	110	110	156	156	180	184	244	244
42	Espanha	41.92037	-8.81136	2016	107	107	158	163	128	138	166	166	186	190	189	189	205	209	253	253	113	121	186	186	208	214	267	267	110	110	152	156	180	180	244	244
43	**Espanha**	**43.66426**	**-7.59453**	**2016**	**99**	**99**	**158**	**163**	**128**	**128**	**160**	**166**	**178**	**180**	**189**	**189**	**209**	**209**	**253**	**253**	**113**	**113**	**182**	**182**	**208**	**208**	**251**	**267**	**110**	**110**	**152**	**156**	**180**	**180**	**242**	**242**
44	Espanha	42.20189	-8.67227	2016	99	107	158	163	138	138	166	166	186	186	189	189	205	209	253	253	137	137	186	186	214	214	251	267	110	110	156	156	184	184	244	244
45	Espanha	42.20189	-8.67227	2016	99	107	158	163	128	128	166	166	180	186	189	189	205	205	253	253	121	137	186	186	208	214	267	267	110	110	156	156	180	180	244	244
48	Espanha	42.22836	-8.45993	2016	99	107	163	163	138	138	166	166	186	186	189	189	205	205	253	253	121	137	186	186	214	214	251	251	110	110	156	156	184	184	244	244
49	Espanha	42.52597	-8.38037	2016	99	99	158	158	128	138	166	166	178	186	189	195	205	205	253	253	113	113	182	186	208	214	251	267	108	110	156	156	180	180	244	244
50	Espanha	42.41052	-8.64912	2016	99	99	158	163	138	138	166	166	180	186	189	189	205	205	253	253	121	121	186	186	214	214	251	267	110	110	152	156	180	184	244	244
51	Espanha	42.66573	-8.18550	2016	107	107	158	163	138	138	160	170	178	180	189	189	205	205	253	253	113	113	182	186	208	208	251	267	108	110	144	156	180	180	242	244
52	Espanha	42.73744	-8.59655	2016	99	107	163	163	138	138	166	166	178	180	189	189	205	205	253	253	113	121	186	190	208	214	251	267	108	110	144	156	180	184	242	244

Table S1: Continued

Sample ID	Country	Coord. Y	Coord. X	Year	R1-36		R1-169		R4-114		D3-15		LIST 2015		LIST 2020B		R4-33		R1-77		LIST 2018B		R1-137		D2-185		VMA-8		R1-80		R1-75		R4-100		VMA-6	
53	Espanha	42.73744	-8.59655	2016	99	107	163	163	138	138	166	166	180	186	189	189	205	205	253	253	113	137	186	186	208	214	251	251	110	110	152	152	180	180	244	244
54	Espanha	42.13430	-8.57061	2016	99	99	158	158	138	138	166	166	186	186	189	189	205	209	253	253	121	121	186	186	214	214	267	267	110	110	152	156	180	180	244	244
55	Espanha	42.11683	-8.54426	2016	99	99	158	163	128	128	166	166	186	186	189	189	205	209	253	253	121	137	186	186	214	214	251	267	110	110	152	152	180	184	244	244
56	**Espanha**	**42.20236**	**-8.18880**	**2016**	**99**	**99**	**163**	**163**	**128**	**128**	**166**	**166**	**180**	**180**	**189**	**189**	**205**	**205**	**253**	**253**	**113**	**113**	**186**	**186**	**214**	**214**	**267**	**267**	**110**	**110**	**152**	**152**	**184**	**184**	**244**	**244**
57	**Espanha**	**42.58160**	**-9.03675**	**2016**	**99**	**107**	**158**	**158**	**128**	**138**	**166**	**170**	**180**	**186**	**189**	**195**	**205**	**209**	**253**	**253**	**137**	**137**	**186**	**190**	**208**	**214**	**267**	**267**	**110**	**110**	**152**	**156**	**180**	**180**	**242**	**244**
58	**Espanha**	**43.27656**	**-8.42347**	**2016**	**99**	**107**	**158**	**163**	**128**	**138**	**160**	**170**	**178**	**180**	**189**	**189**	**205**	**209**	**253**	**253**	**113**	**137**	**182**	**182**	**208**	**208**	**251**	**251**	**108**	**110**	**156**	**156**	**180**	**180**	**242**	**242**
59	Espanha	43.11103	-8.72967	2016	99	107	158	158	128	138	170	170	180	180	189	195	205	205	253	253	113	113	182	182	208	208	251	267	108	110	144	156	180	184	244	244
60	**Espanha**	**43.08192**	**-8.68172**	**2016**	**99**	**99**	**158**	**163**	**128**	**138**	**166**	**170**	**178**	**180**	**189**	**195**	**205**	**209**	**253**	**253**	**113**	**137**	**182**	**186**	**208**	**208**	**267**	**267**	**110**	**110**	**156**	**156**	**180**	**184**	**244**	**244**
61	**Espanha**	**42.91660**	**-9.14847**	**2016**	**99**	**107**	**158**	**163**	**128**	**138**	**166**	**170**	**180**	**180**	**189**	**189**	**205**	**205**	**253**	**253**	**137**	**137**	**182**	**190**	**208**	**208**	**251**	**267**	**108**	**108**	**144**	**156**	**180**	**180**	**244**	**244**
62	Espanha	41.94818	-8.06772	2016	99	99	163	163	138	138	166	166	180	186	189	189	209	209	253	253	137	137	186	186	208	214	251	267	110	110	152	156	180	180	244	244
121	Espanha	41.95339	-8.05608	2016	99	99	158	163	128	138	166	166	186	186	189	189	205	205	253	253	113	121	186	186	214	214	267	267	110	110	156	156	180	184	244	244
122	Espanha	42.08006	-8.06225	2016	99	99	158	163	128	128	166	166	180	186	189	189	205	205	253	253	121	137	186	186	214	214	251	267	110	110	152	156	180	180	244	244
123	**Espanha**	**43.18911**	**-2.05638**	**2016**	**99**	**107**	**158**	**163**	**128**	**138**	**166**	**170**	**178**	**186**	**189**	**195**	**209**	**209**	**253**	**253**	**113**	**137**	**182**	**182**	**208**	**208**	**251**	**267**	**110**	**110**	**156**	**156**	**180**	**180**	**242**	**242**
124	**Espanha**	**43.32547**	**-1.93014**	**2016**	**99**	**99**	**163**	**163**	**128**	**138**	**166**	**166**	**178**	**180**	**189**	**189**	**205**	**205**	**253**	**253**	**113**	**121**	**182**	**190**	**208**	**208**	**251**	**267**	**108**	**110**	**156**	**156**	**180**	**180**	**242**	**242**
125	**Espanha**	**43.33810**	**-1.78885**	**2016**	**107**	**107**	**158**	**158**	**134**	**134**	**160**	**166**	**180**	**180**	**189**	**189**	**205**	**209**	**253**	**253**	**121**	**137**	**182**	**190**	**208**	**214**	**267**	**267**	**108**	**110**	**156**	**156**	**180**	**180**	**244**	**244**
211	Espanha	41.96552	-8.76019	2017	99	107	163	163	128	138	166	166	186	186	189	189	205	209	253	253	121	137	186	186	214	214	251	267	110	110	156	156	180	180	244	244
238	**Espanha**	**41.97971**	**-8.73250**	**2017**	**99**	**107**	**163**	**163**	**128**	**138**	**160**	**160**	**178**	**180**	**189**	**189**	**205**	**209**	**253**	**253**	**113**	**113**	**182**	**186**	**208**	**208**	**267**	**267**	**110**	**110**	**156**	**156**	**180**	**180**	**242**	**242**
280	Itália	43.77922	7.66397	2017	107	107	158	158	128	128	170	170	178	180	189	189	209	209	253	253	137	137	186	190	208	208	251	251	110	110	144	144	180	184	242	244
281	Itália	43.82357	7.74007	2017	99	99	158	158	128	138	160	170	178	178	189	189	205	209	253	253	113	137	186	190	208	214	251	251	110	110	152	152	180	180	242	242
282	Itália	43.85707	7.74007	2017	99	107	158	158	138	138	160	170	178	180	189	189	209	209	253	253	113	137	186	190	214	214	251	251	110	110	152	152	180	184	242	242
296	Itália	43.90234	7.842915	2017	99	107	158	158	138	138	170	170	178	178	195	195	205	209	253	253	113	137	186	190	208	214	251	251	110	110	144	152	180	184	242	242
297	**Itália**	**43.98822**	**7.842915**	**2017**	**99**	**99**	**158**	**158**	**128**	**138**	**160**	**170**	**178**	**180**	**189**	**195**	**205**	**209**	**253**	**253**	**113**	**137**	**186**	**186**	**208**	**208**	**251**	**251**	**110**	**110**	**144**	**152**	**180**	**184**	**242**	**242**
298	**Itália**	**43.82357**	**7.740067**	**2017**	**99**	**107**	**158**	**158**	**128**	**138**	**170**	**170**	**180**	**180**	**189**	**189**	**205**	**209**	**253**	**253**	**137**	**137**	**186**	**190**	**208**	**208**	**251**	**267**	**110**	**110**	**152**	**152**	**180**	**184**	**244**	**244**
301	Itália	43.80008	7.628582	2017	99	99	158	158	128	138	170	170	178	180	189	195	205	209	253	253	113	137	186	190	208	214	251	251	110	110	152	152	184	184	242	244
302	Itália	43.84197	7.662219	2017	99	99	158	158	128	128	170	170	180	180	189	189	205	209	253	253	113	113	186	190	208	214	251	267	110	110	144	144	180	180	242	244
304	Itália	43.81174	7.584586	2017	99	99	158	158	138	138	160	170	180	180	189	195	205	209	253	253	137	139	186	186	214	214	251	251	110	110	152	152	180	180	242	244
306	**Itália**	**43.86964**	**7.549500**	**2017**	**99**	**99**	**158**	**158**	**128**	**128**	**160**	**170**	**178**	**178**	**189**	**195**	**205**	**209**	**253**	**253**	**121**	**137**	**186**	**186**	**208**	**208**	**267**	**267**	**110**	**110**	**156**	**156**	**180**	**180**	**244**	**244**
307	Itália	43.91832	7.784392	2017	99	99	158	158	128	128	170	170	178	180	195	195	205	205	253	253	113	113	186	186	208	214	251	251	108	110	144	152	180	184	242	244

Table S2: Differences in Allelic richness (below diagonal) and unbiased genetic diversity (above diagonal) between populations of the invaded range in Europe and native

	Portugal	**Spain**	**Italy**	**France**	**Vietnam**	**Indonesia**	**China Yunnan**	**China Zhejiang/ Jiangsu**
Portugal		0.0649	0.0649	**0.0008**	**0.0021**	**0.0004**	**2.69E-06**	**7.83E-06**
Spain	0.0186		0.5895	**0.0034**	0.0302	**0.0035**	**4.65E-06**	**1.15E-05**
Italy	0.1093	0.2622		**0.0001**	0.0204	**0.0024**	**3.85E-06**	**1.67E-05**
France	**6.10E-05**	**6.10E-05**	**0.002**		0.985	0.0478	**6.98E-05**	**0.0003**
Vietnam	**0.0004**	0.017	**0.0036**	1.000		0.1934	**0.0014**	0.0065
Indonesia	**6.76E-05**	**0.001072**	**0.0004**	0.0437	0.1934		0.0332	0.1189
China Yunnan	**2.57E-06**	**7.43E-06**	**1.73E-06**	**5.96E-05**	**0.0031**	0.0364		0.5193
China Zhejiang/ Jiangsu	**5.32E-06**	**5.14E-06**	**3.88E-06**	**2.31E-05**	**0.0007**	0.0372	0.8435	

P-values in bold are significant following sequential Bonferroni correction (α = 0.05, k = 28)