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Chloroplast and nuclear genetic diversity and differentiation across European *taxa* of white oaks (*Quercus* L. section *Quercus*)

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La quercia caduta

Dov'era l'ombra, or sé la quercia spande
morta, né più coi turbini tenzona.
La gente dice: Or vedo: era pur grande!
Pendono qua e là dalla corona
i nidietti della primavera.
Dice la gente: Or vedo: era pur buona!
Ognuno loda, ognuno taglia.
A sera ognuno col suo grave fascio va.
Nell'aria, un pianto… d'una capinera
che cerca il nido che non troverà.

Giovanni Pascoli (Primi poemetti, 1907)

The fallen oak

Where the shade once was, the oak itself now spreads,
A corpse, no longer struggling with the wind.
The people say: I see now, it was tall!
So here and there the little nests of spring depend
Upon the branches, from their fallen heights
The people say: I see now, it was a friend!
They all praise, they all cut. At twilight.
They all come and haul their loads away.
Then, in the air, a blackcap's cry.
Seeking its nest, lost for today.

Giovanni Pascoli (Primi poemetti, 1907)

Gestutzte Eiche

Wie haben sie dich, Baum, verschnitten
Wie stehst du fremd und sonderbar!
Wie hast du hundertmal gelitten,
Bis nichts in dir als Trotz und Wille war!
Ich bin wie du, mit dem verschnittnen,
Gequälten Leben brach ich nicht
Und tauche täglich aus durchlittnen
Roheiten neu die Stirn ins Licht.
Was in mir weich und zart gewesen,
Hat mir die Welt zu Tod gehöhnt,
Doch unzerstörbar ist mein Wesen,
Ich bin zufrieden, bin versöhnt,
Geduldig neue Blätter treib ich
Aus Ästen hundertmal zerspellt,
Und allem Weh zu Trotze bleib ich
Verliebt in die verrückte Welt.

Hermann Hesse (Die Gedichte, 1919)

Pruned Oak

Oh oak tree, how they have pruned you.
Now you stand odd and strangely shaped!
You were hacked a hundred times
until you had nothing left but spite and will!
I am like you, so many insults and humiliations
could not shatter my link with life.
And every day I raise my head
beyond countless insults towards new light.
What in me was once gentle, sweet and tender
this world has ridiculed to death.
But my true self cannot be murdered.
I am at peace and reconciled.
I grow new leaves with patience
from branches hacked a hundred times.
In spite of all the pain and sorrow
I'm still in love with this mad, mad world.

Hermann Hesse (Die Gedichte, 1919)

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Abstract

An extensive analysis on the chloroplast and nuclear genetic diversity and differentiation across European *taxa* of white oaks (*Quercus* L. section *Quercus*) was performed. Chloroplast DNA (cpDNA) variation was studied to clarify the biogeography and the history of the populations and to reconstruct genealogical relations between cpDNA haplotypes. Furthermore, a nuclear SSRs analysis was performed to provide data on the genetic structure and diversity and to verify if the groups of oaks analysed were genetically distinct and if their identification was in accordance with the current taxonomic classification. The study was carried out over a vast area of the central and southern Europe, the Balkan Peninsula, and the northern Africa. The sampling design included 11 countries and 1987 individuals (trees) belonging to 93 populations and representing 13 *taxa* of the *Quercus* genus. The genomic DNA of 270 samples was analysed by means of DNA sequence polymorphism at two plastid loci: the *trn*H-*psb*A intergenic spacer and a portion of the *trn*K-*mat*K region. Haplotype diversity, pairwise uncorrected p-genetic distances, number of polymorphic sites, nucleotide polymorphism, nucleotide diversity, and number of parsimony informative characters in the two marker regions were evaluated. Moreover, genealogical relations between cpDNA haplotypes were reconstructed. The nuclear microsatellite markers analysis was performed on a subset of 1072 individuals belonging to 58 populations. The patterns of the genetic structure and the hierarchical structure of the sampled populations were assessed. In addition, genetic diversity and differentiation within and among populations were estimated and analysis of molecular variance (AMOVA) and Principal Coordinates Analysis (PCoA) were performed. The phylogeographic analysis based on the study of the chloroplast DNA variation allowed to confirm the relatively recent colonization of the section *Quercus* in Eurasia and to document the little differentiated plastome of this oaks group, as shown by the low degree of diversity found in the fourteen haplotypes identified in the studied area. While the detected nucleome variability did not reflect the considerable morphological variability responsible for the many ecomorphotypes and the taxonomic confusion in the European white oaks group. Based on the obtained results, five genetically distinct groups, among the twelve *taxa* identified based on morphological features, were recognized, so the taxonomic classification for the European white oaks was not always confirmed by molecular analyses. This study demonstrated that both the young history of the European white oaks and the repeated events of hybridisation and introgression are responsible for a group that is not still genetically fixed and resolved.

Keywords: *Quercus*, White Oaks, Chloroplast DNA, Haplotype, Nuclear SSRs, Marker, Genealogical Relations, Genetic Structure

1 Introduction

1.1 The cultural and symbolic importance of the *Quercus* genus in human societies

Emblem of longevity, strength, stability, endurance, fertility, power, justice, and honesty, oaks have been a part of traditional rites and celebrations for many centuries, since the early Proto-Indo-European societies (Chassé 2016; Leroy et al. 2020). Regarded as symbolic representations by human cultures, political structures, and religions all over the northern hemisphere, they were also associated with gods as Zeus, Jupiter, and Thor in the mythology of ancient Greece, Rome, and Germany (Leroy et al. 2020).

Based on the very earliest connections between humans and trees, symbolism may have been associated with trees in early human cultures. Furthermore, in comparison with other tree species, oaks guided the colonization of the Middle East and Europe by *Homo sapiens* between 45000 and 50000 years ago (Bar-Yosef and Belfer-Cohen 2013). In fact, the remnants of *Quercus ithaburensis* and *Q. calliprinos* acorns in caves inhabited by humans indicate that oaks were a valuable food resource for humans during the early colonisation of Eurasia (Lev et al. 2005; Leroy et al. 2020). According to Kremer (2015), there is a strong link between the colonization dynamics of oaks with human populations invasion of Europe during the Upper Paleolithic and human migration in response to postglacial warming. The extensive use of oaks was in fact related to their application not only for food purposes, but also as source of fuel, medicine, shelter, or for artistic creation (Anderson 2007).

1.2 Origin and diversification of the *Quercus* genus

Based on fossilized pollen records, it is estimated that oaks arose about 56 million years ago, at the Paleocene / Eocene boundary (Hofmann 2010; Hofmann et al. 2011), when temperatures were 10°C warmer than today (Zachos et al. 2001). The earliest evidence of the *Quercus* genus dates to the Late Paleocene, as is shown by the finding of pollen grains of *Quercoidites* at the St. Pankraz site, in Austria (Hofman et al. 2011). Moreover, several palynological records which date back to the Eocene were found in North America (Bones 1979; Manchester 1994), China (Jiang 1993), and Europe (Kvaček and Walther 1989).

Numerous hypotheses and scenarios have been advanced on the geographic origin of the *Quercus* genus and the migration routes followed by the first lineages, however data currently available do not provide clear evidence and to date all the assumptions remain uncertain and unconfirmed. Nevertheless, it is thought that the *Quercus* genus underwent extremely rapid diversification as a result of significant climatic changes during the Oligocene and Miocene (Trelease 1924; Axelrod 1983), attaining an almost modern distribution in the Neogene (Barron et al. 2017).

According to Zhou (1992), the *Quercus* genus had origin from the probable ancestor *Trigonobalanus*, during the Paleogene in southeastern Asia, and subsequently migrated towards Europe and America by two routes: via the North Atlantic Land Bridge before the Eocene, and via the Bering Land Bridge after the Miocene. While, according to Manos and Stranford (2001) the *Quercus* genus originated from the boreal tropical deciduous forest that occupied the northern hemisphere at the beginning of the Tertiary and then differentiated with the separation of the continents. For these temperate *taxa*, an initial divergence between North American and Asian clades and the dispersion of section *Quercus* of the North American clade to Europe was supposed. Moreover, floristic exchange via both North Atlantic Land Bridge and the Bering Land Bridge, was assumed. Hubert et al. (2014) instead, hypothesized a first divergence within the *Quercus* genus at the Paleocene / Eocene boundary and the first radiation within 'New World Clade' and 'Old World Clade' by the end of the middle Eocene. Furthermore, they supported a migration via the North Atlantic Land Bridge until at least 8 Ma (Denk et al. 2010) and via Bering Land Bridge until the latest Pliocene or Pleistocene interglacials. According to Denk et al. (2017), based on the pollen records of white oaks from the middle Eocene in western Greenland and the Baltic amber region of northern Europe, can be assumed that the early radiation of the white oaks involved the North Atlantic Land Bridge. In addition, presence of data from Eocene and Oligocene strata in East Asia suggested a migration from North America via the Bering Land Bridge. Therefore, the modern western Eurasian roburoids had origin as a result of the spread of this East Asian branch of white oaks (early roburoids).

The paleobotanical records show that the oaks underwent a basal vicariance between Eurasia and North America (Manos and Stanford 2001; Hipp et al. 2019) and that since the early diversification of *Quercus*, the genus was well differentiated, and geographically structured clades were recognizable (Fig. 1.1). The main lineages, currently attributable to the sections recognized by Denk et al. (2017) *Cyclobalanopsis*, *Lobatae*, *Quercus*, and possibly *Ilex*, originated within 10 million years, after the early Eocene climatic optimum, and over the past 40 million years, as consequence of the decrease of the temperatures, they differentiated mainly *in situ* (Axelrod 1983; Manos and Stanford 2001), diversifying ecologically within each region (Kremer and Hipp 2020). Even, section *Protobalanus* had origin during the Eocene in North America, where it remained limited until the present. Furthermore, in the mid-late Eocene, oaks were spread in the territory of the former USSR, and it is thought that *Quercus* (mainly *Q. pseudoneriifolia*) had a crucial role in these ecosystems. During the late Eocene, sections *Quercus*, *Lobatae*, and *Protobalanus*, as well as the ring-cupped oaks were spread in North America, while in Europe section *Lobatae* and the subgenus *Cyclobalanopsis* occurred (Barron et al. 2017).

Both in North America and Europe, increases in diversification rates were driven by ecological opportunities, but while differentiation happened in sympatry in North America, this was not recorded in Europe. More in detail, in Americas a northern ancestor originated white and red oaks, belonging to the sections *Quercus* and *Lobatae*, that then diversified southward (Zachos et al. 2001), radiating in western and eastern North America, and subsequently in Mexico and central America (Hipp et al. 2018; Cavender-Bares 2019; Kremer and Hipp 2020). While in Europe, at first the sections *Cerris* and *Ilex* diversified ecologically within clades (Hipp et al. 2019). Subsequently, between 10 and 20 million years ago, the *Roburoid* white oak clades, originated by an eastern North American ancestor, arrived in Eurasia, and in lack of related oaks diversified at a higher rate than the *Cerris* and *Ilex* sections (Kremer and Hipp 2020). Sclerophyllous evergreen oaks *Cerris* and *Ilex* were important components of the pre-Mediterranean vegetation of central and southern Europe during the Neogene. Moreover, from the Pliocene to the end of Neogene, even subgenus *Cyclobalanopsis* was spread in Europe, although it dominated mainly forests in southeastern Asia, where a lower presence of sections *Ilex* and *Quercus* also occurred. The group *Cyclobalanopsis* was documented even in North America probably by the late Paleogene (Barron et al. 2017). To date, it is present only in East Asia (Denk et al. 2017).

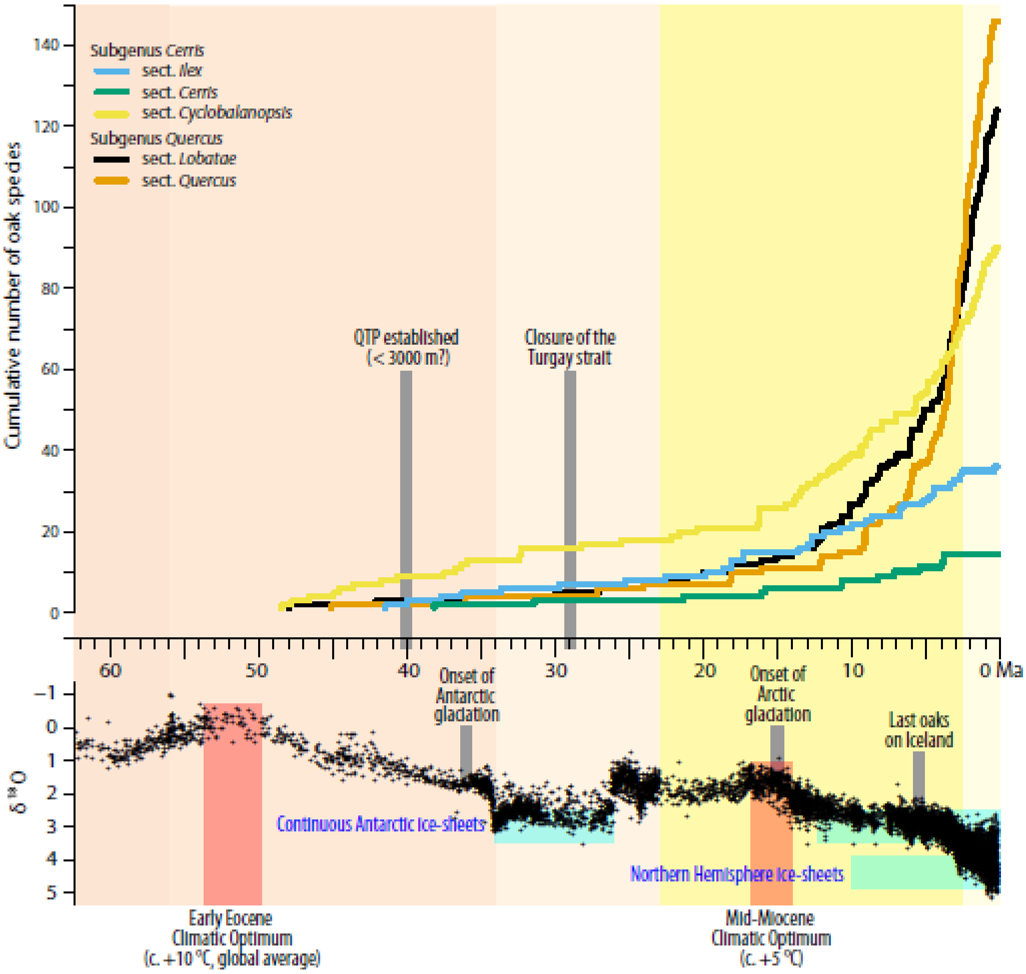


Figure 1.1 Lineages through time plot from Hipp et al. (2020) displaying the diversification of the sections *Cerris*, *Cyclobalanopsis*, *Ilex*, *Lobatae*, and *Quercus*. For comparison, tectonic events in the northern Hemisphere and global climate context are referred. Cenozoic epochs (following Walker et al. (2018) (Paleocene, Eocene, Oligocene, Miocene, Quaternary)) are shown in different colours in the background

During the quaternary era, large portions of Europe and North America were periodically covered by the continental ice shield (Zweck and Huybrechts 2005), and this considerably affected the oak's geographic range in the northern hemisphere. In response to these climate fluctuations, oaks have been subjected both to widespread extinction and important migrations (Yan et al. 2019). During the last glacial period, the temperate forest species were confined to the glacial refugia, restricted area with a milder climate (Brewer et al. 2002). In Europe the main southern refugia were Iberian Peninsula, southern Italian Peninsula, and the southern Balkan Peninsula (Petit et al. 2002b). While in North America, oaks retreated to the unglaciated southeastern portion of the continent (Delcourt and Delcourt 1981; Delcourt and Delcourt 1984).

The postglacial recolonization took place from the late glacial (15000-10000 years ago) to the beginning of the Holocene (10000 years ago), when the climate was more stable and favourable to deciduous trees species, and species started to leave the refugia and shift northward from them. In Europe, oaks first spread to the central European mountains in the late-glacial interstadial (13000-11000 years ago), then, during the Holocene, they expanded into northern

Europe, quickly into the North West, and more slowly into the centre and East, due to physical barriers (Brewer at al. 2002).
Both the climate fluctuations of the glacial period and bottleneck effects during interglacial and postglacial recolonization greatly influenced and reduced the genetic diversity of the oaks (Yan et al. 2019). Moreover, while the oak's distribution range was firstly conditioned by the shifts in climate, subsequently competition between species, landscape topography and other edaphic factors controlled mainly the distribution (Brewer et al. 2002).

To date, the genus *Quercus* L. (Fagaceae) is among the most widespread and species rich tree genera in the northern hemisphere (Kremer and Hipp 2020). It consists approximately of 500 species (Nixon 1993; Manos et al. 1999), and it covers wide areas from the equator (Colombia and Indonesia) up to the boreal regions in Europe, and from sea level to 4000 m a.s.l. in China (Yunnan province) (Camus 1936, 1938, 1952; Menitsky 2005; De Beaulieu and Lamant 2010; Kremer and Hipp 2020). The northern limit of dispersal of the oaks in Eurasia is between 62° and 63° northern latitude in Scandinavia, where only *Quercus robur* and *Q. petraea* can grow, while the southern limit is in the Sunda platform, a few degrees South of the Equator (Menitsky 2005). In America the northern limit of distribution is at 50° northern latitude, and the southern limit is in Colombia, where only *Quercus humboldtii* Bonpl. occurs. The majority of the species are distributed in the eastern part of the country, up to the Mississippi river, and few oak species reach Canada (*Quercus garryana*, *Q. rubra*, *Q. alba*, and *Q. macrocrapa*) (Manos 2016). In addition, the distribution of oaks space in a variety of habitats from bottomland forests to open grasslands and savannas, and from alkaline to acidic soils (Kremer and Hipp 2020). The highest number of species and the greatest diversity is documented in Mexico and East Asia (Valencia-Avalos 2004; Menitsky 2005; Nixon 2006).

1.3 The taxonomic classifications of the *Quercus* genus

Due to the large number of species, vast geographic distribution, presence of intermediate morphological traits mainly caused by extensive hybridization (Rushton 1993; Cavender-Bares et al. 2004; Curtu et al. 2007; Burgarella et al. 2009; Moran et al. 2012), introgression, incomplete lineage sorting, and convergent evolution (Kole 2011), the taxonomy of the *Quercus* genus is particularly complex. Moreover, as the variation within species is so high, several authors have questioned the concept of species (Burger 1975; Van Valen 1976) suggesting numerous classifications for the genus. To date, the main classifications of the *Quercus* genus have been based entirely on morphological characters (Denk et al. 2017).
Reviewing the treatment of the *Quercus* genus over time, the first classification was proposed by Carl von Linné in *Species Plantarum* (Linné 1753), in which fourteen species of oaks were documented in Europe and North America. The list of the species included white oaks (*Quercus alba*, *Q. aesculus* (= *Q. petraea* (Matt.) Liebl.), *Q. robur*, and *Q. prinus* (status unresolved)), red oaks (*Q. rubra*, *Q. nigra*, and *Q. phellos*), *Cerris* oaks (*Q. cerris*, *Q. aegilops* (= *Q. macrolepis* Kotschy), *Q. suber*), and *Ilex* oaks (*Q. ilex*, *Q. coccifera*, *Q. gramuntia* (= *Q. ilex*), and *Q. smilax* (= *Q. ilex*)) (Denk et al. 2017).
Between 1787 and 1816, Carl Ludwig Willdenow described twenty species, among which there was *Quercus pubescens* Willd. (1805), still undefined.

Subsequently, John Loudon (1838, 1839), based on the classification proposed by Carl von Linné, revised the *Quercus* genus describing 150 species and distinguishing ten sections. In addition to the eight sections identified considering the species described by Carl von Linné, Loudon recognized the sections *Virentes* (southeastern North American) and *Lanatae* (Nepal). Moreover, he proposed the subdivision of the European oaks in the sections *Cerris, Ilex,* and *Robur* corresponding to the three major clades resulting from the recent molecular-phylogenetic trees (Denk et al. 2017).

The recognition of the Asian group of oaks *Cyclobalanopsis* is attributed to Anders Ørsted, who in 1871 identified in the subtribe *Quercinae*, two different genera: *Quercus* and *Cyclobalanopsis*. According to his classification, five subgenera with a total of 16 sections and about 184 species were described in the *Quercus* genus.

The classification of the American oaks was deepened by William Trelease (1924), who listed about 371 species belonging to three subgenera, represented by: *Leucobalanus* (white oaks), *Erythrobalanus* (red oaks), and *Protobalanus* (intermediate oaks). This grouping defined the division of the *Quercus* genus in three sections in the Americas, sections *Quercus*, *Lobatae,* and *Protobalanus* (Jensen 1997; Nixon and Muller 1997).

Afterward two different systems of classification represented from the monographic work of Aimée Antoinette Camus (1936-1938, 1938-1939, 1952-1954) and the classification of Otto Karl Anton Schwarz (1936), respectively, were followed in western/southern Europe and in central/eastern Europe, correspondingly. More in detail, in Camus classification the *Quercus* genus was subdivided in two subgenera: subgenus *Euquercus* (*Quercus s.s.*), divided into six different sections, and subgenus *Cyclobalanopsis*, spread in South Asia (Camus 1936-1954).

While, Schwarz (1936) divided *Quercus* into four separate genera: *Cyclobalanopsis* and *Erythrobalanus* belonging to *Cyclobalanopsideae*, and *Macrobalanus* and *Quercus*, belonging to *Querceae*.

In 1984, Yuri Leonárdovich Menitsky distinguished three subgenera represented by *Cyclobalanopsis*, *Quercus*, and *Heterobalanus* and classified the *Ilex* oaks into the subgenus *Heterobalanus*, while *Cerris* oaks formed with *Quercus* one of the two sections in subgenus *Quercus* (Denk et al. 2017).

Numerous other classifications were proposed between 1878 and 1986 by several other authors (for instance, Nyman, Boissier, Borzì, Lojacono, De Candolle, Ascherson and Graebner, Reichinger, Krüssmann).

In 1993, Kevin Nixon proposed a classification in which distinguished two subgenera in the *Quercus* genus, represented by the subgenus *Cyclobalanopsis*, including the cycle-cup oaks and the subgenus *Quercus*, including all the remaining oaks. More in detail, in the latter subgenus, three sections were distinguished: two natural sections represented by the section *Lobatae*, comprising the red oaks and the section *Protobalanus*, comprising the intermediate oaks, and an artificial, heterogeneous, northern hemispheric section *Quercus* including all white oaks, *Cerris*, and *Ilex* oaks (Denk et al. 2017).

The classification schemes proposed from Loudon to Nixon, following the reconstruction proposed by Denk et al. (2017), are shown in Figure 1.2.

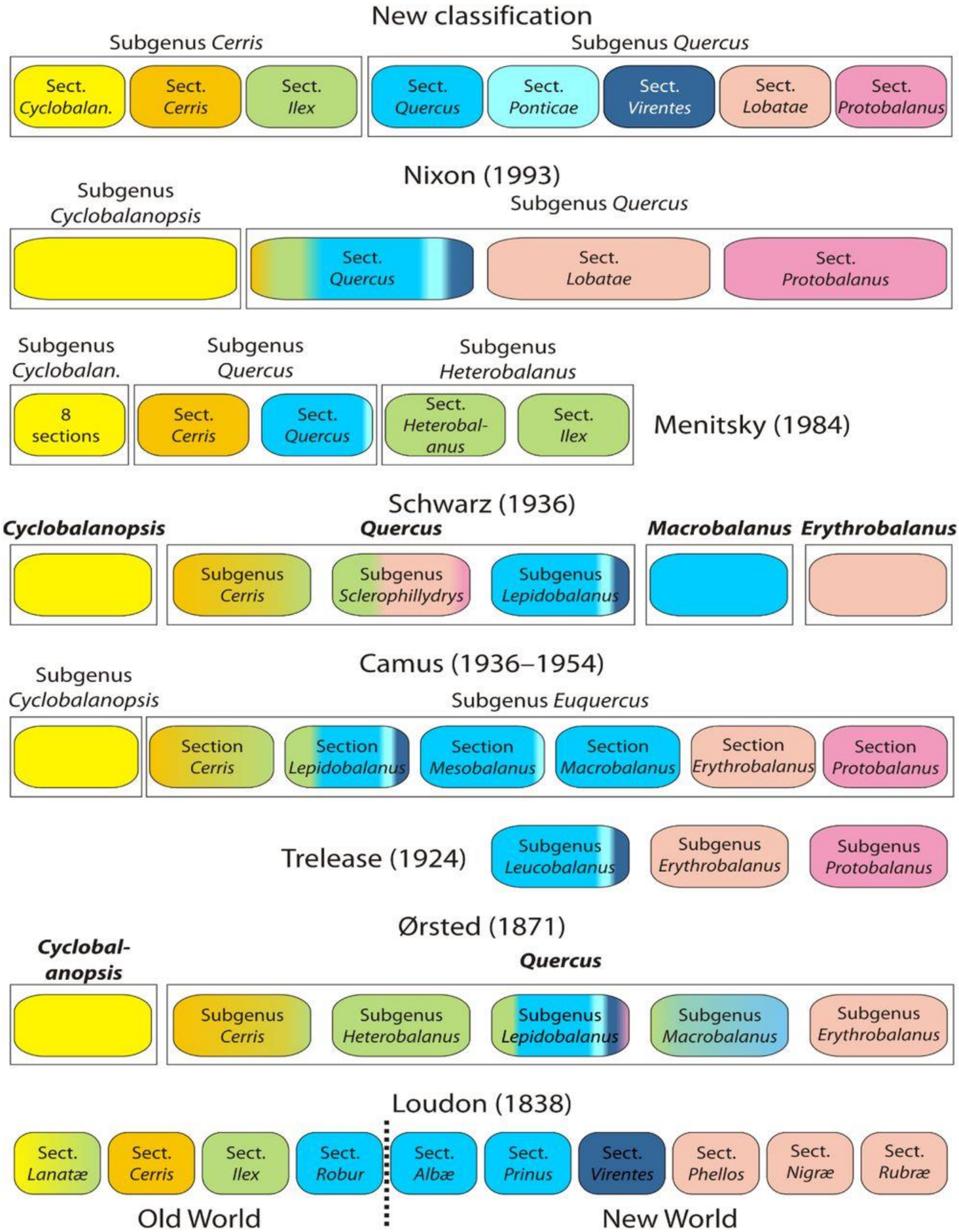


Figure 1.2 Classification schemes for the *Quercus* genus from Loudon to Nixon. The systematic affiliation of species included in each *taxon* is represented by the colour coding. Section *Cyclobalanopsis* (cycle-cup oaks, yellow), section *Cerris* (*Cerris* oaks, orange), and section *Ilex* (*Ilex* oaks, green) for the ‘Old World clade’; section *Quercus* (white oaks *s.s.*, blue), section *Virentes* (cyan), section *Ponticae* (dark blue), section *Protobalanus* (intermediate oaks, purple), and section *Lobatae* (red oaks, red) for the ‘New World clade’ (From Denk et al. 2017)

In the early 2000's, the availability of DNA markers and sequences provided new tools to solve the taxonomical classification problem, and molecular analyses replaced or complemented the morphological approaches in the taxonomic studies.

Manos et al. (2001) proposed the first molecular phylogeny of *Quercus* based on sequences of the nuclear ITS region and plastid RFLP data. In their classification, the family of Fagaceae was divided into two subfamilies: *Fagoideae* (*Trigonobalanus s.l.*) and *Castaneoideae* (*Castanea*, *Castanopsis*, *Chrysolepis*, *Lithocarpus*, and *Quercus*). Three clades were identified into the *Quercus* genus: subgenus *Cyclobalanopsis* including subtropical to tropical South-East Asian species; New World species classified within subgenus *Quercus* representing sections *Quercus s.s.* (*sensu* Manos et al. 1999), *Protobalanus*, and *Lobatae* (*sensu* Nixon 1993, 1997); Old World species previously classified within subgenus *Quercus* section *Cerris* (Camus 1936-1954; Denk et al. 2017).

Subsequently, phylogenetic reconstructions were further improved with molecular data and several new classifications of the *Quercus* genus were proposed. Five or six major groups were recognised on the basis of molecular analyses (Manos et al. 2001; Oh and Manos 2008; Denk and Grimm 2010): the North American group *Protobalanus*, the groups *Lobatae* and *Quercus*, the Eurasian groups *Cerris* and *Ilex* (according to Denk and Grimm 2010) and the East Asian group *Cyclobalanopsis*. A larger collection of nuclear-encoded genes was subsequently used to confirm these six infrageneric groups (Hubert et al. 2014).

The most recent classification is attributable to Denk et al. (2017) that, based on pollen morphology, position of aborted ovules, DNA sequence data (nuclear encoded genes) and nuclear reduced representation of next-generation sequencing (RADseq) data set, recognized two reciprocally monophyletic groups of oaks, *Quercus* and *Cerris*, with eight phylogenetic sections. These groups strongly supported the old dichotomy between oak subgenera that dates to the early Eocene (Manos and Hipp 2021). Moreover, the two subgenera highlight the important biogeographic split among modern *taxa* (Fig. 1.3), as the subgenus *Quercus* comprises three American endemic sections, *Lobatae*, *Protobalanus*, and *Virentes*, and two Holarctic North American and Eurasian sections, *Ponticae* and *Quercus*, while the subgenus *Cerris* includes three sections, *Cerris*, *Ilex*, and *Cyclobalanopsis*, all exclusively occurring in Europe, Asia, and northern Africa (Manos and Hipp 2021).

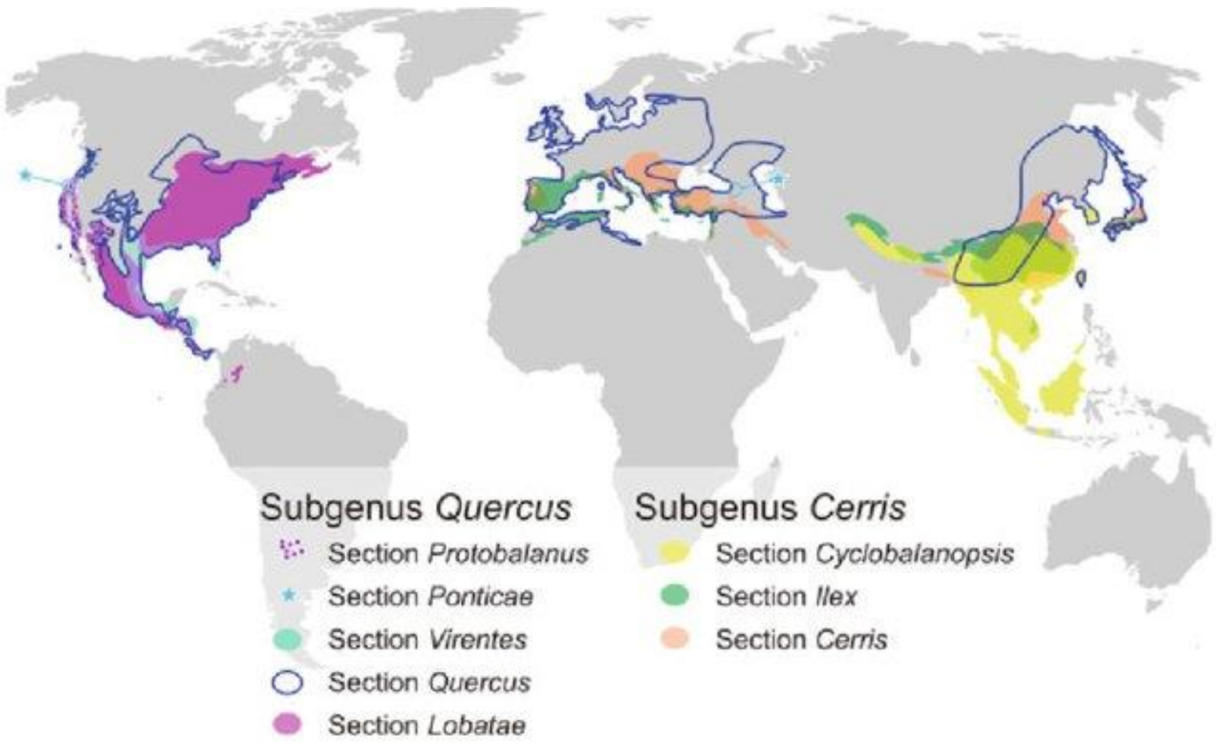


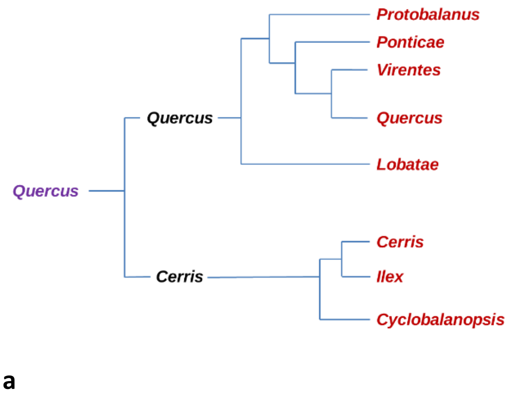
Figure 1.3 Geographic distribution of the eight sections of the *Quercus* genus (From Denk et al. 2017)

1.3.1 The eight sections of the *Quercus* genus

The identified groups according to Denk et al. (2017), can be summarised as follows (Fig. 1.4 a/b):

- ✓ Subgenus ***Quercus*** (the 'New World clade' (Manos et al. 2001) or 'high-latitude clade' (Grímsson et al. 2015; Simeone et al. 2016)):
    - section *Protobalanus* (= Trelease's subgenus of the same name): the North American intermediate oaks
    - section *Ponticae*: the western Eurasian and western North American oaks
    - section *Virentes*: the American "southern live oaks"
    - section *Quercus*: all white oaks from North America (= Trelease's subgenus *Leucobalanus*) and Eurasia (= Menitsky's section *Quercus*)
    - section *Lobatae* (= Trelease's subgenus *Erythrobalanus*): the North American red oaks

- ✓ Subgenus ***Cerris*** (the exclusively Eurasian 'Old World clade' or 'mid-latitude clade'):
    - section *Cerris* (= Menitsky's section *Cerris* plus *Quercus suber*): the *Cerris* oaks
    - section *Ilex* (= Menitsky's subgenus *Heterobalanus* minus *Quercus suber*): the *Ilex* oaks
    - section *Cyclobalanopsis* (former subgenus *Cyclobalanopsis* of Ørsted, Camus, Schwarz, Menitsky, and Nixon): the cycle-cup oaks of East Asia (including Malesia)



Quercus L.	Sections	No. of Species
subgenus *Quercus*		297
	Protobalanus (Trel.) Schwarz	5
	Ponticae Stef.	2
	Virentes Loud.	7
	Quercus	150
	Lobatae Loud.	120
subgenus *Cerris* Oerst.		138
	Cerris Dumort.	13
	Ilex Loud.	35
	Cyclobalanopsis (Oerst.) Benth. & Hook. f.	90

b

Figure 1.4 a Phylogeny of the clades of the *Quercus* genus according to the classification of Denk et al. (2017). **b** Number of species within each infrageneric group

In the ‘New World clade’ (*Quercus* subgenus *Quercus*) approximately 297 species are enumerated.

Section *Protobalanus* includes 5 species (*Quercus cedrosensis* C.H. Muller, *Q. chrysolepis* Liebm., *Q. palmeri* (Engelm.) Engelm., *Q. tomentella* Engelm., *Q. vacciniifolia* Kellogg) and represents the only evergreen American group. According to Manos et al. (1999) and Ortego et al. (2018) a North-South discontinuity in California, perhaps related to an ancient disjunction that limited seed dispersal is documented. This divergence between the southern and northern lineages dates to 5 million years ago.

Section *Ponticae* is represented by two species, *Quercus pontica* (western Eurasia) and *Q. sadleriana* (northwestern America). Despite their disconnected geographic distribution both species have comparable morphologies and occupy similar latitudes and ecological areas that overlap in the mean values of 19 climatic variables (Fick and Hijmans 2017). For this section, it is supposed an American origin followed by dispersal to Europe, given the nested phylogenetic location of *Ponticae* within the American oak clade. According to Manos (2016), divergence could have happened between 16 and 20 million years ago. Despite the lack of clear macrofossil evidence, *Ponticae* are relicts of a once more widespread species group (Denk et al. 2017).

Because of its consistent and distinctive morphologic features, section *Virentes* has long been regarded as one of the most clearly defined species groups of the American oaks. Resulting in all the phylogenomic trees sister to the section *Quercus*, its relationship to the white oaks is still not resolved (Manos and Hipp 2021). The restricted distribution and historical migration patterns of this section are likely affected by the group's vulnerability to freezing temperatures and drought tolerance. To date it is possible suppose a temperate origin in North America, followed by East-West dispersion and subsequent migration South, that dates from 11 to 23 million years ago (Denk and Grimm 2009).

Section *Lobatae* ranges in a variety of habitats in Americas, and it is the only section to reach South America, where *Quercus humboldtii* Bonpl. occurs in northern Colombia (Valencia-Avalos 2004; Nixon 2006). An early diversification of red oaks in North America, followed by a southward shift to Mexico, associated with an increased rate of diversification, is dated around 15 million years ago (Hipp et al. 2018).

Section *Quercus* represents the most species rich group (about 150 species) and shows the greatest diversity of geographic distribution across the clades. It is spread in North America, Mexico, central America, western Eurasia, East Asia, and North Africa (Nixon and Muller 1997). The highest diversity occurs in Mexico, where approximately 100 species are found (Nixon 2006). Analyses of the timing and biogeography of *Quercus* indicate an initial diversification of white oaks in North America, followed by a concomitant movement southward, and then across the full geographic range of Eurasia. Although it is still unclear whether the direction of movement into Eurasia was West through the Beringian Land Bridge or East through the North Atlantic Land Bridges, according to recent analyses based on whole plastome genome data a clear origin of the roburoids within the Americas can be supposed (Manos and Hipp 2021).

In the ‘Old World clade’ (*Quercus* subgenus *Cerris*) approximately 138 species are enumerated.

Section *Cerris* (*Cerris* oaks) comprises 13 species of deciduous or semi-evergreen trees, distributed in a variety of habitats from the Atlantic coasts of the Iberian Peninsula and Morocco to Japan, and under a variety of climates (Köppen-Geiger climate types), including cold steppe and warm temperate or snow climates with different precipitation regimes (Kottek et al. 2006; Rubel et al. 2016; Simeone et al. 2018). This section appears to be a relatively recent offshoot of the group *Ilex* based on fossil records from both groups and the results of dating by Hubert et al. (2014).

Section *Ilex* comprises about 35 evergreen species widely distributed in Mediterranean Europe, northern Africa, and the East Asian subtropics (Menitsky 1984; Denk and Grimm 2010; Deng et al. 2017; Denk et al. 2017). Fossils of section *Ilex* characterised the Cenozoic Tethyan forest flora of Europe, the Himalayas, and East Asia (Guo 1978; Li and Guo 1976; Xu et al. 1973).

Section *Cyclobalanopsis* includes approximately 90 species, mainly distributed in tropical and subtropical humid areas of Asia (Deng 2007; Denk and Grimm 2010). These species are adapted to warm, humid climates and are often dominant trees in evergreen broad-leaved forests (Deng et al. 2018). The higher diversity is observed in southwestern China and northern Indo-China, where approximately 60 species are known to occur (Luo and Zhou 2000). Fossil *taxa* of the section *Cyclobalanopsis* have been found in East Asia since the Oligocene, with numerous occurrences in the Neogene in East Asia and on the southern Himalayan slope (Jia et al. 2015; Xu et al. 2016).

1.4 The current state of knowledge on the sections of the *Quercus* genus

To date all sections of the *Quercus* genus have been studied from different perspectives and by means of various approaches (morphology, genetics, ecology). However, the availability of integrated and complete data still remains lacking, mainly for some sections and/or geographic areas. While American and Asian studies have produced complete genome sequencing for many *taxa* of the genus (e.g., Hubert et al. 2014; Liu et al. 2019; Zhang et al. 2020; Zhou et al. 2022) and have reconstructed in detail the genealogical relations between plastid variants at large geographic scales, in Europe the availability of similarly extensive studies is extremely limited. Moreover, the lack of comparative and large-scale analyses and the availability of numerous localized studies increase, leaving them unresolved, the critical issues that distinguish the genus.

1.4.1 Knowledge gap in the genealogical relations between plastid DNA variants in the *taxa* of the European white oaks (section *Quercus*)

Analyses on the chloroplast genome variability have been carried out on all sections of the *Quercus* genus in order to investigate the evolutionary history and to reconstruct genealogical relations between plastid DNA variants (e.g., Manos et al. 1999; Olalde et al. 2002; Petit et al. 2002a; Petit et al. 2002b; Zhang et al. 2015; Yang et al. 2017). Due to the highly conserved structure, general recombination-free, small effective population sizes (Birky et al. 1983), as well as the uniparental (maternal) inheritance, that permits to examine the dispersal routes (Dumolin et al. 1995), the cpDNA proves to be extremely efficient in phylogenetic studies. Understanding the geographic and environmental processes that influence spatial genetic diversity patterns is also important to predict species responses to future climatic conditions and preserve diversity (Bay et al. 2017, 2018). Climate change may represent a challenge for the tree species, indeed despite their propensity for local adaptation, trees are susceptible to rapid environmental change due to their long generation times and immobility (Petit and Hampe 2006; Yan et al. 2019).

Detailed knowledge on the chloroplast genome variability is currently available for the majority of sections of the *Quercus* genus, however in the Euro-Mediterranean region, recent and updated reconstructions are available only for the sections *Ilex* and *Cerris* (e.g., Simeone et al. 2013, 2016, 2018), while for the section *Quercus*, the last studies date back to the early 2000s (Petit et al. 2002a, 2002b; Fineschi et al. 2002).

1.4.2 Knowledge gap in the taxonomy of the European white oaks (section *Quercus*)

Although to date sufficiently robust taxonomic classifications of the *Quercus* genus have been proposed in a global context, the classification of European oaks remains unresolved, especially for many *taxa* belonging to the section *Quercus*. The manifestation of hybrid forms is often the major cause of the taxonomic confusion and represents a challenge for the proper classification of some *taxa*. Hybridization and introgression are indeed well-documented phenomena in the *Quercus* genus and are significant sources of genetic and morphological variability (Fortini et al. 2015). Even though hybridization and introgression between species from different sections are rare in nature, due to the reproductive isolation (Hubert et al. 2014), their occurrence within sections is extremely frequent (Hipp 2015), and manifestation of this phenomenon among *taxa* of white oaks such as *Quercus robur* L., *Q. petraea* (Matt.) Liebl., and *Q. pubescens* Willd. is well recorded (Dupouey and Badeau 1993; Bussotti and Grossoni 1997; Fortini et al. 2009, 2022; Viscosi et al. 2011, 2012; Di Pietro et al. 2016, 2021). Crosses commonly take place in mixed forest where different oak *taxa* cohabit, moreover the high degree of phenotypic plasticity that distinguishes this genus further increases the diversity that results (Fortini et al. 2015). As a consequence, *taxa* identification can frequently be inaccurate or misleading (Jensen et al. 1993; Ponton et al. 2004). The most critical issues characterize the pubescent oaks group, especially in Italy. In fact, although this group has been treated by several authors, for many *taxa* it is still not possible to recognize diagnostic characters that support the status of the species. An example are the pubescent oak *taxa* considered typical of the flora of southern Italy, such as *Quercus amplifolia* Guss., *Q. congesta* C.Presl, *Q. dalechampii* Ten., *Q. ichnusae* Mossa, Bacc. & Brullo, *Q. leptobalanos* Guss., and *Q. virgiliana* (Ten.) Ten., that while being accepted in important Italian Floras (Pignatti 1982; Brullo et al. 1999; Pignatti 2017-2019), are not unanimously recognized in international institutions (e.g., The Plant List; IPNI; Euro + Med PlantBase), as well as in phytosociological syntheses (Brullo et al. 2001; Bacchetta et al. 2009; Biondi et al. 2010). In fact, in the Portal to the Flora of Italy, according to the morphological and molecular studies carried out in Italy over the past 15 years, some *taxa* are not regarded valid, and they are considered synonymous with *Quercus pubescens*. Differently, for other European white oaks as *Quercus robur* L., *Q. petraea* (Matt.) Liebl., and *Q. frainetto* Ten., despite the presence of dubious cases due to gene flow phenomena, occurring particularly in presence of *Q. pubescens* individuals, an easier identification can be made using the traditional morphological characters and molecular approaches (Fineschi et al. 2002; Curtu et al. 2007; Fortini et al. 2009; Viscosi et al. 2011).

The difficulties in matching the taxonomic and nomenclatural classifications proposed by Floras and Checklists with a corresponding clustering based on morphological, and/or genetic characters represent a crucial issue (Fortini et al. 2022) as it is proved by several studies (Bordács et al. 2002; Bruschi and Grossoni 2004; Škvorc et al. 2005; Franjic et al. 2006; Fortini et al. 2009; Viscosi et al. 2011; Enescu et al. 2013; Curtu et al. 2015; Di Pietro et al. 2016, 2021; Proietti et al. 2021). In light of these considerations, the use of genetic analyses to support the identification based on morphological traits of the *taxa*, turns out to be fundamental to fill the gaps in knowledge currently available, mostly for the European white oaks.

1.4.2.1 Analysis of the genetic diversity by means of simple sequence repeat markers

Due to their high levels of polymorphism, widespread distribution across most plant genomes, and ease of use, the microsatellites or simple sequence repeats (SSRs) have become one of the most popular molecular markers for plant genotyping in the last 20 years (Mason 2015) and provide an ideal tool to examine and detect genetic variation patterns within populations (Ekué et al. 2009).

SSRs are a type of repetitive DNA sequence ubiquitous in most plant genomes and contain repeats of a motif sequence 1-6 bp in length. Due to these features, SSRs frequently undergo mutations, primarily as a result of errors in DNA polymerase, which involve the addition or subtraction of a repeat unit. SSRs can be identified in expressed sequence tags (ESTs) and more frequently in nongenic DNA sequences because they occur in both genic and nongenic regions and are occasionally transcribed (Mason 2015). Due to their location in coding or closely linked regions, EST-SSR markers have a high transferability rate among *taxa* (Ellis and Burke 2007) and can be used for the study of local adaptation (Müller and Gailing 2018).

1.5 Aims of research

To fill the gaps in the knowledge currently available on the *taxa* of white oaks of the central southern Europe, belonging to the section *Quercus* of the *Quercus* genus and to improve the knowledge about evolutionary history and the taxonomic classification, the following study was carried out. The main aims of the research were:

I. to reconstruct genealogical relations between plastid DNA variants
II. to capture rare genetic variants that are important because they could subtend divergent evolutionary lineages
III. to provide data on the genetic structure and diversity
IV. to verify if the groups of oaks analysed were genetically distinct and if their identification was in accordance with the current taxonomic classification.

1.6 *Taxa* involved in the research

The *taxa* of white oaks (*Quercus* L. section *Quercus*) analysed in the current study include: *Q. banatus* P. Kucera, *Q. congesta* C. Presl, *Q. dalechampii* Ten., *Q. faginea* Lam., *Q. frainetto* Ten., *Q. ichnusae* Mossa, Bacch. & Brullo, *Q. leptobalanos* Guss., *Q. petraea* (Matt.) Liebl., *Q. petraea* subsp. *austrotyrrhenica* Brullo, Guarino & Siracusa, *Q. pubescens* Willd., *Q. pyrenaica* Willd., *Q. robur* L., and *Q. virgiliana* (Ten.) Ten.

1.6.1 *Quercus banatus* P. Kucera

Quercus banatus P. Kučera (= *Q. dalechampii* sensu auct. medioeur non Ten.) is a tree widely distributed in the central Europe and Balkan Peninsula (Fig. 1.5).

It reaches Poland in its northern distribution (Požgaj and Horváthová 1986; Koblížek 1993; Magic 2006) and Caucasus eastward (Magic 2006). In the previous literature sources, *Quercus dalechampii* Ten. was the name given to oak populations of *Q. banatus*. However, after the extensive review of the application of the name *Quercus dalechampii* Ten. proposed by Di Pietro et al. (2012), the name *Q. dalechampii* Ten. was used to designate oaks belonging to the pubescent oak type (group of *Q. pubescens* Willd.). Therefore, Kučera (2019) proposed a name for *Quercus dalechampii* sensu auct. medioeur. non Ten., namely *Q. banatus* P. Kučera (based on *Q. aurea* Wierzb. ex Kotschy; syn. *Q. aurea* Wierzb.), following Schwarz's (1936) synonymization of "*Q. dalechampii* Ten." with *Q. aurea* Wierzb. (Schwarz 1937; Beldie 1952; Pignatti 1982; Magic 2006; Kučera 2019).

Figure 1.5 Leaves of *Quercus banatus* P. Kučera (From Kučera 2019)

1.6.2 *Quercus congesta* C. Presl

Quercus congesta C. Presl is a deciduous tree up to 20 m tall, with greyish-brown bark, longitudinally and transversely furrowed, divided into elongated scales. The young twigs are pubescent and greyish. The petiole is 0.4-1.4 cm long. The leaves are leathery, elliptic to oblong, and subcordate at base, 5-8.5 cm in length and 3-5 cm in width, with maximum width in the middle part. The upper page of the leaves is sparsely hairy and green, the lower page is densely pubescent and greyish in colour. The leaves have 5-8 shallow lobes per each side, variously rounded, sometimes mucronate. The male catkins are 4-8 cm in length and female catkins are 3-8 floriferous. The acorns are ovoid to ellipsoid, rounded and mucronate at apex, 2-3 cm long and 1-1.5 cm in diameter. The acorn cup, usually covering up to 1/4 of the acorn, is ciaziform, regular-edged, with oblong-lanceolate embriciate scales having rounded to suboptuse brown apex, flat on dorsum, finely pubescent at base and sparsely pubescent in the upper part (Fig. 1.6).

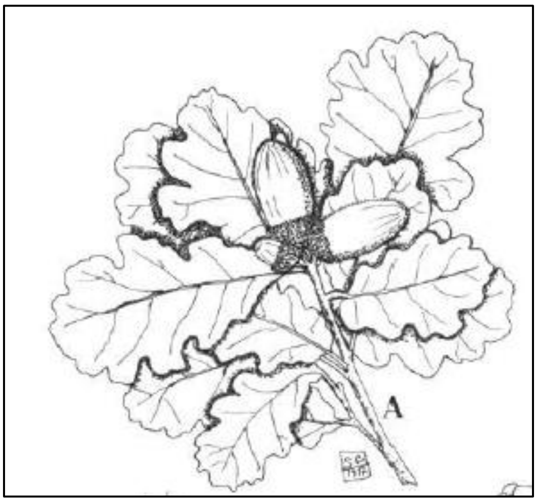


Figure 1.6 Leaves and acorns of *Quercus congesta* C. Presl (From Brullo et al. 1999)

Flowering period is in April and the fruits mature in October. *Quercus congesta* occurs in northern Sicily, southern Calabria, and Sardinia (Brullo et al. 1999) (Fig. 1.7).

Figure 1.7 Distribution map of *Quercus congesta* C. Presl (From Brullo et al. 1999)

1.6.3 *Quercus dalechampii* Ten.

The name *Quercus dalechampii* Ten. has been applied to different *taxa* in the past, as the European botanists followed two different positions on the taxonomic relationships of *Q. dalechampii*. The first, proposed by Schwarz (1936-1939, 1964), regarded *Quercus dalechampii* as a species belonging to the *Q. petraea* species complex, while the second, supported by Camus (1936-1954) and, more recently, by Brullo et al. (1999), considered *Q. dalechampii* as belonging to the *Q. pubescens* species complex (Di Pietro et al. 2012). The misunderstanding in the application of the name *Quercus dalechampii* Ten. is due to the incongruence between the original description of *Q. dalechampii*, indicating leaves (abaxial surfaces) and young twigs glabrous (typical of the *Q. petraea* complex), and most of the original material which has leaves (abaxial surfaces) and young twigs pubescent (typical of the *Q. pubescens* complex). In 2012, Di Pietro et al. solved the problem of an inconsistent application of the name *Quercus dalechampii* Ten. by designating a lectotype which explicitly belongs to the pubescent oak type (group of *Q. pubescens* Willd.). Following this revision, the name *Quercus dalechampii* Ten. cannot be applicable to the populations in central Europe that were previously categorized under it, as well as to the populations in southeastern Europe (Beldie 1952; Gančev and Bondev 1966; Kučera 2019). The lectotype designated is from southern Italy (Gioia Tauro (Reggio), Calabria). According to this lectotypification, the name *Quercus dalechampii* Ten. is to be applied to a species belonging to the *Q. pubescens* complex, having oblong and deeply lobed leaves, sparsely pubescent on the adaxial side and pubescent on the abaxial side, with an acute apex and a subcordate base. Twigs are pubescent, fruits are sessile with a cupule having slightly tomentose and rough scales (Di Pietro et al. 2012) (Fig. 1.8).

Figure 1.8 Lectotype of *Quercus dalechampii* Ten. (From Di Pietro et al. 2012)

Therefore, when interpreted as a species taxonomically related to *Quercus petraea* and reported from different countries in southeastern Europe, all records of *Q. dalechampii* are most likely to represent *Q. petraea s.l.* or another *taxon* (Di Pietro et al. 2012).

1.6.4 *Quercus faginea* Lam.

Quercus faginea Lam., also known as the Portuguese oak, is a medium-sized deciduous or semi-evergreen tree (up to 20 meters tall). The bark is greyish-brown. The twigs are pubescent or glabrescent. The leaves are ovate-oblongate, toothed or lobed, with teeth or lobes up to the base and are 3-15 cm long and 1.5-9 cm wide (Castroviejo 1986-2012) (Fig. 1.9).

Figure 1.9 Leaves of *Quercus faginea* Lam.

The petiole, pubescent or glabrescent, is 5-20 mm in length. The flowering period of this species is between March and April. The oblong-ovoid acorns are 2-2.5 cm long and the acorn cup has ovate triangular scales. The Portuguese oak is a species native to the western Mediterranean region, and it is spread in Algeria, Morocco, Portugal, Spain, and Tunisia (Castroviejo 1986-2012) (Fig. 1.10).

Figure 1.10 Distribution map of *Quercus faginea* Lam. (From PoWO)

It occurs from sea level to 1900 m a.s.l. and it is a basophile *taxon* that grows in different soils, from sub-humid to humid ombrotypes, or in temporihygrophile positions in drier stations (Castroviejo 1986-2012).

1.6.5 *Quercus frainetto* Ten.

Quercus frainetto Ten., commonly known as Hungarian oak, is a deciduous tree, that rarely lives longer than 200 years and grows to heights of more than 30 m. Although it occasionally exists as an introduced species in Hungary, this oak is not endemic to this country, despite its name (Bartha 1998). The twigs and the lower surface of the leaves are covered with dense hairs. The leaves are large and up to 25 cm long, widest close to the apex, with many deep-cut lobes (Mitchell et al. 1974), and an ear-like base (Mauri et al. 2016) (Fig. 1.11).

Figure 1.11 Leaves of *Quercus frainetto* Ten.

The flowers are monoecious and are wind pollinated. The egg-shaped acorns can grow up to 25 mm in length and the acorn cup is sessile and covered with long overlapping scales and hairs (Clinovschi 2005; Johnson and More 2006). High fructification rates occur roughly every 5-8 years (Jerković and Marijanović 2010).

The Hungarian oak is an element of the sub-Mediterranean flora (Corcuera et al. 2002) and is thought to be native to southeastern Europe (Bartha 1998), with the Balkan Peninsula having the widest spread. It is also present in northwestern Turkey and southern Italy (Meusel and Jager 1989) (Fig. 1.12).

Figure 1.12 Distribution map of *Quercus frainetto* Ten. (From PoWO)

Quercus frainetto is a meso-xerophilous species, light demanding and cannot tolerate shading (Chatziphilippidis and Spyroglou 2006). It can grow in heavy acidic soils and tolerates some water logging (Sanders et al. 2014). This species occurs in pure stands, but more commonly it coexists with other species like hop hornbeam (*Ostrya carpinifolia*), oriental hornbeam (*Carpinus orientalis*), South European flowering ash (*Fraxinus ornus*), and Turkey oak (*Quercus cerris*) (Bartha 1998).

1.6.6 *Quercus ichnusae* Mossa, Bacch. & Brullo

Quercus ichnusae Mossa, Bacchetta & Brullo is an endemic *taxon* to Sardinia and owes its name to the old name of the Island, "Ichnusa". It is mainly widespread in the central and northwestern part of Sardinia (Fig. 1.13).

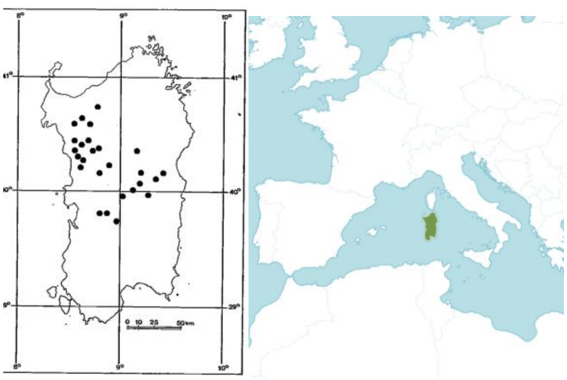


Figure 1.13 Distribution map of *Quercus ichnusae* Mossa, Bacchetta & Brullo (From Mossa et al. (1999) and PoWO)

Quercus ichnusae is a deciduous tree (up to 25 m tall), with a pale, brown-greyish, and irregularly furrowed bark. The twigs, as well as the leaves, are pubescent. The leaves are oblong to oblong-obovate, 6-15 cm in length and 2.8-9 cm in width, with 4-7 lobes per side generally mucronate at the apex. The staminate catkins are grouped and the pistillate catkins are solitary or grouped (2-3). The acorn cup covers 1/3-2/3 of the acorn and is regular and hemispherical with a peduncle of 5-20(30) mm in length. It is covered by imbricate scales. The proximal ones are ampulliform to pyriform, strongly gibbous dorsally, subglabrous, or sparsely pubescent, and the distal ones are linear-elliptical, densely pubescent, finely imbricate, forming a wide margin. The acorn is dark brown, ovoid-ellipsoid rounded at the apex with a short appendage (Fig. 1.14).

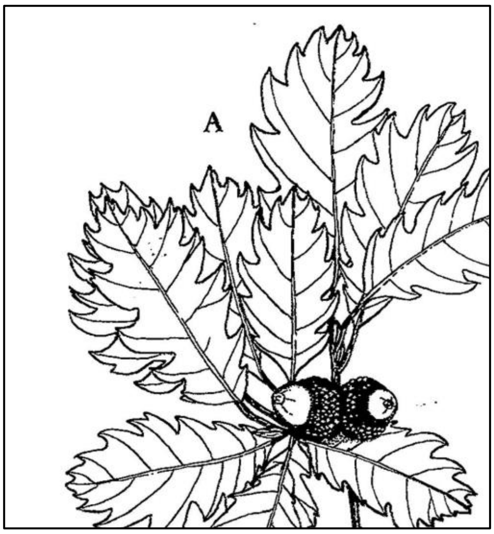


Figure 1.14 Leaves and acorns of *Quercus ichnusae* Mossa, Bacchetta & Brullo (From Mossa et al. 1999)

Quercus ichnusae flowers in March and the production of acorns is in October. It grows on fresh and deep soil and on siliceous substrata and occurs in the meso-supramediterranean belt, at 400-1200 m a.s.l. *Quercus ichnusae* grows both in pure woods and with other *taxa*, such as *Q. dalechampii*, *Q. congesta*, and *Q. amplifolia* (Mossa et al. 1999).

1.6.7 *Quercus leptobalanos* Guss.

Quercus leptobalanos Guss. is a deciduous tree up to 20 m tall, with greyish bark, longitudinally and transversely furrowed, divided into elongated scales. The young twigs are pubescent and greyish in colour. The petiole is 0.5-1.8 cm long. The leaves are leathery of variable size, 5-15 cm in length and 3.5-11 cm in width, rounded or subcordate at base, with upper page sparsely hairy and dull green, and lower page densely pubescent and greyish, showing 5-7 incisions per side of variable depth, with rounded lobes (Fig. 1.15).

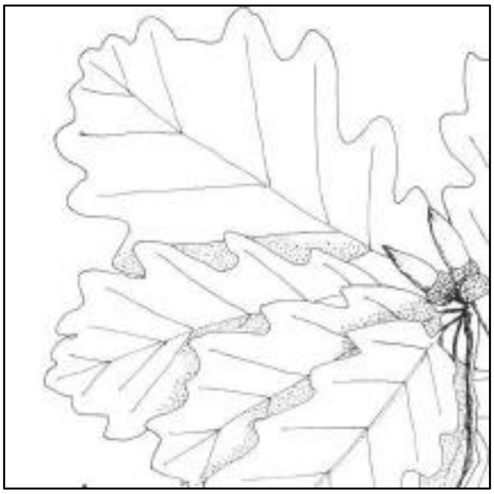


Figure 1.15 Leaves and acorns of *Quercus leptobalanos* Guss. (From Brullo et al. 1999)

Male catkins are 3-5 cm long and female catkins are 3-7 floriferous. Fruit axis greatly elongated, brings 3-7 subsessile or briefly pedunculate fruits. The acorn is ovoid to ellipsoid, rounded and mucronate at apex, 1-3 cm long. Acorn cup, usually covering 1/4 to 1/2 of the acorn, is 0.8-1 cm in diameter, hemispherical to ciaziform, regular-edged, with embriciate scales. The scales are ampulliform, slightly convex on back and furrowed at apex. *Quercus leptobalanos* normally flowers in May and the fruits mature in September. This *taxon* occurs on sandy-textured soils derived from siliceous substrates, between 800 and 1200 m a.s.l. It is spread in the northwestern Sicily, limited to the Madonie mountain and the Ficuzza Wood, near Palermo (Brullo et al. 1999) (Fig. 1.16).

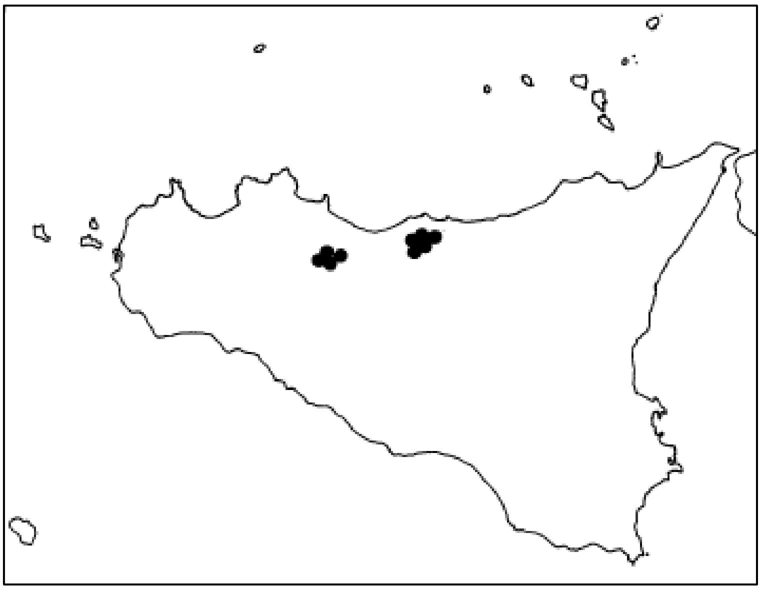


Figure 1.16 Distribution map of *Quercus leptobalanos* Guss. (From Brullo et al. 1999)

1.6.8 *Quercus petraea* (Matt.) Liebl.

Quercus petraea (Matt.) Liebl., also known as sessile oak, is a tree up to 35(50) m tall, with columnar trunk and a smooth silvery bark at the beginning, which at maturity turns brownish, with deep, long longitudinal fissures. The young twigs are greyish-brown and glabrous. Petiole is 1-2.5 cm long. Leaves are oblong to oboval, 8-12(-14) cm in length and 5-7(-11) cm in width, with maximum width in middle part. They are pale green and slightly pubescent at the beginning, while at maturity glabrous and lucent on the upper page and glabrous or with slight pubescence along the main veins on the lower page, presenting (4-)6-7(-8) pairs of rounded lobes, obtuse at the apex (Fig. 1.17).

Figure 1.17 Leaf of *Quercus petraea* (Matt.) Liebl.

The male catkins are 3-5 cm long and the female catkins are 1-5 floriferous. The fruit axis brings 1-5 fruits. Acorn is subellipsoid to cylindrical oblong, rounded and mucronate at apex, 1.5-4 cm long, 1-2.5 cm in diameter. *Quercus petraea* flowers from late February to April and the fruits mature in September. This species grows in more Atlantic climates on light and well-drained, often rocky, soils (hence the specific Latin name *petraea* = of rocky places), generally occurring on slopes and hill tops, and preferring a more acid soil pH. It forms acidophilous woodlands more or less pure or mixed with other deciduous oaks (*Quercus robur*, *Q. humilis*, *Q. cerris*) and beech. *Quercus petraea* is widely distributed in Europe, from the Iberian Peninsula to Russia, and northwestern Turkey (Hedge and Yaltrik 1982). In Italy it is frequent throughout the peninsula as far as Calabria, excluding Sicily (where *Quercus petraea* subsp. *austrotyrrhenica* Brullo, Guarino & Siracusa occurs) and Sardinia (Pignatti 1982) (Fig. 1.18).

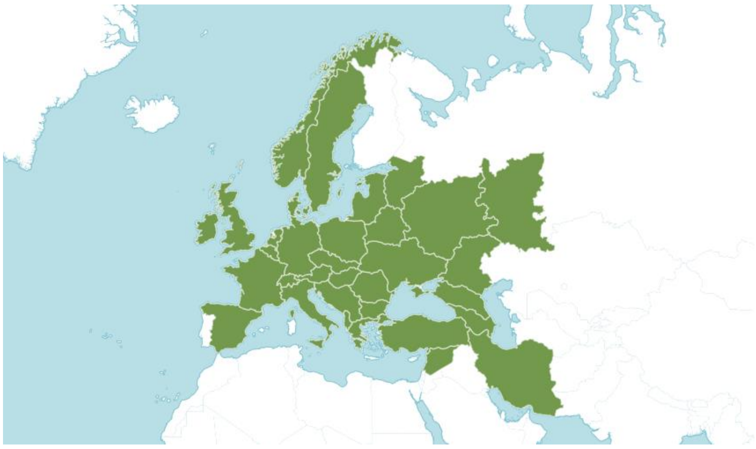


Figure 1.18 Distribution map of *Quercus petraea* (Matt.) Liebl. (From PoWO)

1.6.9 *Quercus petraea* subsp. *austrotyrrhenica* Brullo, Guarino & Siracusa

Quercus petraea subsp. *austrotyrrhenica* Brullo, Guarino & Siracusa is a deciduous tree up to 30 m tall, with columnar trunk, showing brown bark, longitudinally furrowed. The young twigs are glabrous, reddish in colour with prominent lenticels. Petiole is 1-2.5 cm long. Leaves are variable in shape, subrounded to oblong, 8-13 cm in length and 6-11 cm in width, with maximum width in the central part. They are rounded or subcordate at the base, dark green, with upper page lucid, and lower page glabrous or pubescent along the main veins and have 4-5 rounded lobes. Male catkins are 3-6 cm in length, while female catkins are 3-7 floriferous. Fruit axis brings 3-7 acorns. The acorn, oblong to ellipsoid, rounded and mucronate at apex, is 2-3.5 cm long and 1-1.5 cm in diameter. The acorn cup covers 1/3 of the fruit and is subhemispherical to ciaziform, regular-edged, with embriciate, triangular-ampulliform scales, that are gibbous in the lower half, glabrous or subglabrous at the apex, and pubescent at the margin (Fig. 1.19).

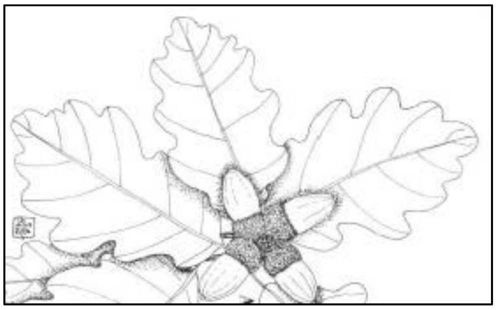


Figure 1.19 Leaves and acorns of *Quercus petraea* subsp. *austrotyrrhenica* Brullo, Guarino & Siracusa (From Brullo et al. 1999)

Quercus petraea subsp. *austrotyrrhenica* flowers in late May-early June and the fruits mature in September. It occurs in the montane plane at elevations between 1100 and 1700 m a.s.l., on schists and gneisses and on fresh and deep, but not excessively moist soils. This subspecies constitutes pure mesophilic woods and occurs exclusively in Sicily, on the Madonie and Nebrodi Mountains, and in Calabria on the massifs of Aspromonte and Serre Calabre (Fig. 1.20).

Figure 1.20 Distribution map of *Quercus petraea* subsp. *austrotyrrhenica* Brullo, Guarino & Siracusa (From Brullo et al. 1999)

Subspecies *austrotyrrhenica* shows an allopatric distribution in comparison to the type, occupying the southernmost stations of the species range. It has clearly differentiated morphologically as a result of the geographic isolation of its populations (Brullo et al. 1999).

1.6.10 *Quercus pubescens* Willd.

Quercus pubescens Willd., commonly known as downy or pubescent oak, is a middle-sized (15-20 m tall) deciduous or semideciduous tree, which owes its name to the leaves densely pubescent. The leaves are alternate, ovate-oblongate, (3)5-10 cm long, with 5-6 more or less deep lobes (Krüssmann 1978). They are green-greyish when develop and turn leathery and dark green when the hairs on their upper side fall out (Fig. 1.21). The petiole is short (5 to 20 mm) (Pasta et al. 2016).

Figure 1.21 Leaves of *Quercus pubescens* Willd.

Pubescent catkins of grouped male flowers (6-10 stamens) grow up at the base of new shoots. The female flowers have greenish stigmata and develop on the axil of distal leaves. The fruits, mostly elliptic (2-3.5 cm long) and enclosed for 1/4 to 1/2 of their total length by cupules covered by imbricate triangular hairy scales, have a short hairy petiole, and are grouped in clusters of 3-4 acorns. The flowering period, which lasts from March to May, differs with altitude. From September to November, the ripen acorns mature and are able to germinate immediately and to develop vigorous roots in very short time (Tonioli et al. 2001). Deep furrows, rough, and thick plates that are distinctive to the bark of downy oaks serve as an effective barrier against grazing fires (Pasta et al. 2016).

As a result of the high morphological variability of the populations of *Quercus pubescens*, many ecomorphotypes spread in the major Mediterranean islands and/or South European peninsulas are considered endemics or exclusive to limited area (e.g., *Quercus amplifolia* Guss., *Q. congesta* C. Presl, *Q. dalechampii* Ten., *Q. ichnusae* Mossa, Bacc. & Brullo, *Q. leptobalanos* Guss., and *Q. virgiliana* (Ten.) Ten.). To date, as the genetic analyses still fail to differentiate these *taxa* (Franjić et al. 2006; Enescu et al. 2013) they are clustered in the *Quercus pubescens s.l.* group. *Quercus pubescens s.l.* has a wide geographic range occupying almost all central and southern Europe from western Spain (Franco 1991; Rivas-Martìnez and Sáenz-Laìn 1991) to the Balkan area, Black Sea, and Caucasus (Pasta et al. 2016) (Fig. 1.22).

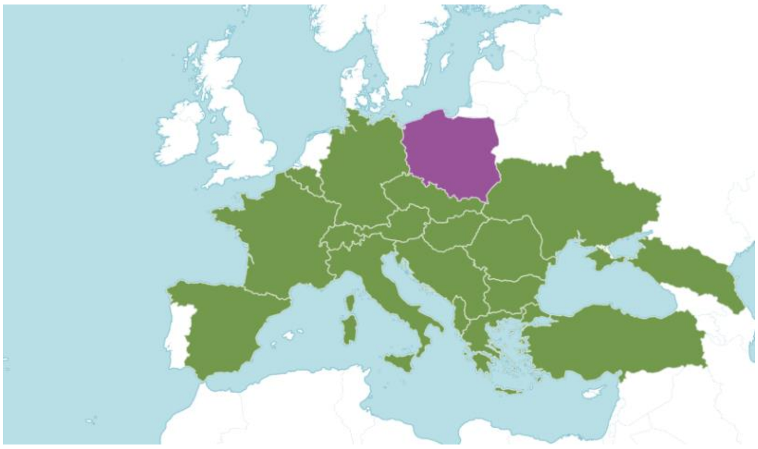


Figure 1.22 Distribution map of *Quercus pubescens* Willd. In green the countries where the species is native and in violet the countries where it is introduced (From PoWO)

Although *Quercus pubescens* is more common on hillsides between 200 and 800 m a.s.l., it grows from coastal plains up to 1200-1300 m a.s.l., mainly in lime-rich and well drained soils in the northern part of their range, while it is common on acidic soils in the warmer countries (e.g., Sicily and Crete). It exhibits both thermophilic and heliophilic behaviours and is suited to both moderate summer drought stress and low winter temperatures. Moreover, while in central Europe, *Quercus pubescens* is restricted to warm microclimatic conditions, *Quercus pubescens*-dominated forests are quite widespread in southern Europe (Pasta et al. 2016). Downy oaks coexist with other species of southeastern Europe and southwestern Asia, such as oriental hornbeam (*Carpinus orientalis*), nettle trees (*Celtis* spp.), manna ash (*Fraxinus ornus*) and hop hornbeam (*Ostrya carpinifolia*) (Pasta et al. 2016).

1.6.11 *Quercus pyrenaica* Willd

Quercus pyrenaica Willd., syn. *Quercus toza* Bosc, also known as Pyrenean oak, is a medium-sized deciduous oak (up to 20 m tall), with an average longevity of 300 years (López Lillo and López Santalla 2007). Although it is not distributed in the Pyrenees, both the scientific and common name of this oak refer to the Pyrenees, as Willdenow named this oak on the basis of dry samples received with a wrong sticker that described its origin in the Pyrenees (López González 2007). The trunk is straight and slender with a thick and cracked greyish-brown bark. The leaves are simple and alternate, with 4-8 pairs of irregular and deep lobes and they are covered by stellate hairs on sides (Franco 1991; López Lillo and López Santalla 2007; López González 2007; Lorite et al. 2008). They are 7-20 cm in length and 4 to 10 cm in width (Fig. 1.23).

Figure 1.23 Leaves of *Quercus pyrenaica* Willd.

The Pyrenean oak is a monoecious tree. The female flowers are grouped in small clusters, while the male catkins appear early summer and are wind pollinated (Johnson and More 2006). The oblong acorns, about 4 cm in length and 1-2.5 cm in width, mature in autumn and are dispersed principally by birds and micro-mammals (López Lillo and López Santalla 2007). Pyrenean oak is spread from western and southwestern France to the Iberian Peninsula (Spain and Portugal) and northern Morocco (Lorite et al. 2008). Since Spain and Portugal represent about 95 % of its natural distribution area, the species can almost be considered as endemic to the Iberian Peninsula (Calabuig et al. 2000) (Fig. 1.24).

Figure 1.24 Distribution map of *Quercus pyrenaica* Willd. (From PoWO)

Quercus pyrenaica ranges from sea level to 2100 m a.s.l. in Sierra Nevada (Franco 1991). This oak inhabits transitional regions between sub-humid temperate and Mediterranean semi-arid conditions and is a typical sub-Mediterranean mountainous species. Its limited growth season affects its distribution (Hernández-Santana et al. 2008). It is a sciaphilous species, and it appears mainly on the mountainside and foot of siliceous mountains, rarely in limestone and dolomites, and prefers loose soils and a sandy texture (López González 2007). *Quercus pyrenaica* usually grows in closed forest, although depending on the level of management its overall appearance can show different degrees of openness, including dehesa (Velasco Aguirre 2014).

1.6.12 *Quercus robur* L.

Quercus robur L., also known as pedunculate or English oak, is a deciduous broadleaved tree, spread in Europe, that can live up to 1000 years and reaches hights of 40 m. The bark is grey, fissured with rectangular elongate blocks. The leaves are simple, obovate-oblong, and deeply and irregularly lobed, with a short petiole (2-7 mm). This species is monoecious and wind pollinated. The male catkins are yellow, 5 cm long, and the female catkins are globular (Mitchell et al. 1974). The acorns, which grow in pairs, are held on the ends of long stems, and are rounded with longitudinal olive-green stripes that are evident when fresh (Fig. 1.25).

Figure 1.25 Leaves and acorns of *Quercus robur* L.

Quercus robur occurs widely across most of Europe, reaching the Norwegian coast and the northern Scotland, in Mediterranean areas it is also present in Portugal, Greece, and South Turkey, and eastwards into continental central Russia, up to the Urals (Ducousso and Bordacs 2003) (Fig. 1.26).

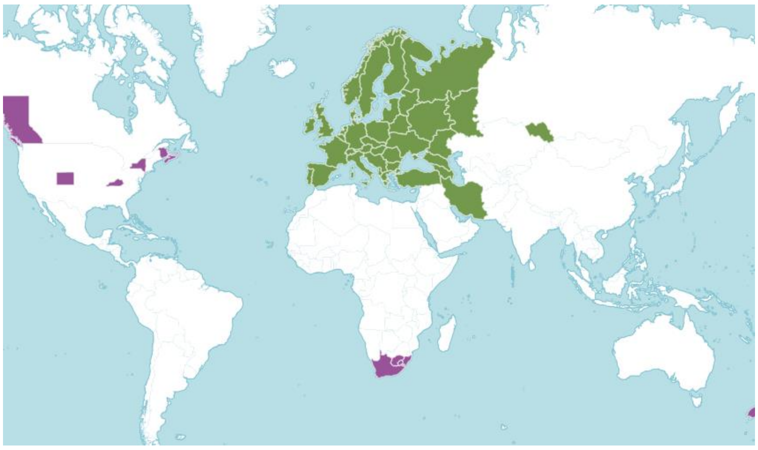


Figure 1.26 Distribution map of *Quercus robur* L. In green the countries where the species is native and in violet the countries where it is introduced (From PoWO)

It grows up to 1300 m a.s.l. in the Alps, in fertile and moist soils. This oak is a light demanding species and occurs in damp to wet and nutrient-rich soils, where it cohabits principally with hornbeam (*Carpinus betulus*) and other deciduous tree species such as ash (*Fraxinus excelsior, Fraxinus angustifolia*), maple (*Acer campestre, Acer platanoides*) and small-leaved lime (*Tilia cordata*) (Eaton et al. 2016).

1.6.13 *Quercus virgiliana* (Ten.) Ten.

Quercus virgiliana (Ten.) Ten. is a deciduous tree up to 20 m tall, with greyish bark, longitudinally and transversely furrowed, divided into elongated scales. The young twigs are pubescent and greyish in colour. The leaves are leathery, 5-8.5 cm in length and 3-5 cm in width, with 3-7 rounded lobes, sometimes mucronate. The acorn is ovoid to ellipsoid, rounded and mucronate at apex, 2-3.5 cm in length. The acorns are grouped in clusters of 1-5. Acorn cup usually covering 1/4 or 1/2 of the acorn, is irregular and has fringed edge due to the prolongation of the scales, that are imbricated and hairy (Fig. 1.27).

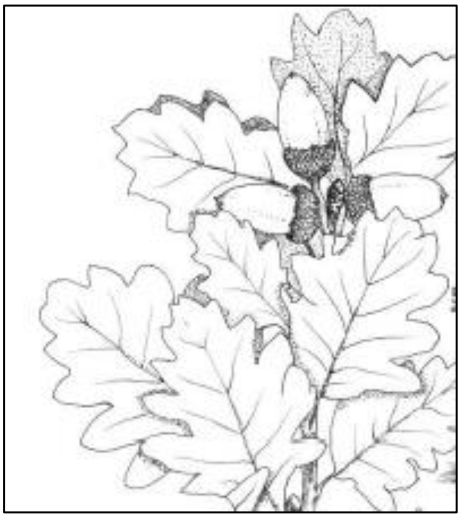


Figure 1.27 Leaves and acorns of *Quercus virgiliana* (Ten.) Ten.

This *taxon* normally flowers in February-March, however individuals found at elevations above 800 m may delay flowering until May. The fruits mature between October and November. *Quercus virgiliana* is found from sea level up to about 1300 m a.s.l. Although, it was thought from several authors that *Quercus virgiliana* was restricted to the coastal plain and the hilly belt, differently from *Q. pubescens*, regarded as a species typical of inland areas and higher altitudes, according to Fortini et al. (2022) no evidence for this assumption were found. *Quercus virgiliana* occurs in mixed evergreen and deciduous oak forests with a predominantly thermophilic character. The presence of the species has been documented in Sicily, Sardinia, and peninsular Italy (Brullo et al. 1999) (Fig. 1.28). According to Borhidi (1995), it is also present in the Balkan area and, it reaches as far as southern Hungary.

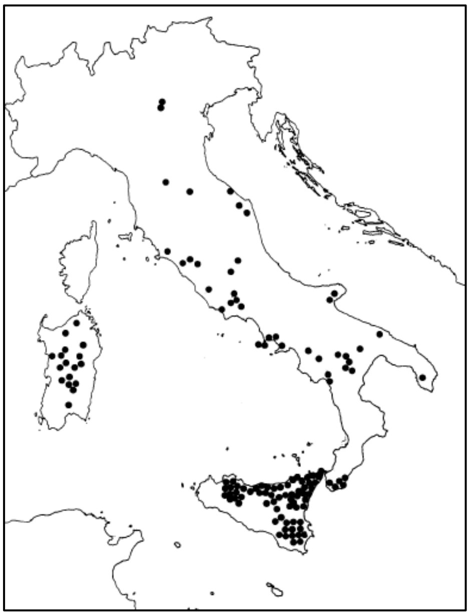


Figure 1.28 Distribution map of *Quercus virgiliana* (Ten.) Ten. in Italy (From Brullo et al. 1999)

2 Materials and methods

2.1 Study area

The study was carried out over a vast area of the central and southern Europe, the Balkan Peninsula, and the northern Africa. Eleven countries were involved in the sampling: Austria, Bulgaria, Croatia, Czech Republic, France, Greece, Italy, Morocco, Romania, Serbia, Spain. From each country a different number of populations was considered and different *taxa* of the *Quercus* genus, section *Quercus* were sampled (Fig. 2.1; Supplementary File 1).

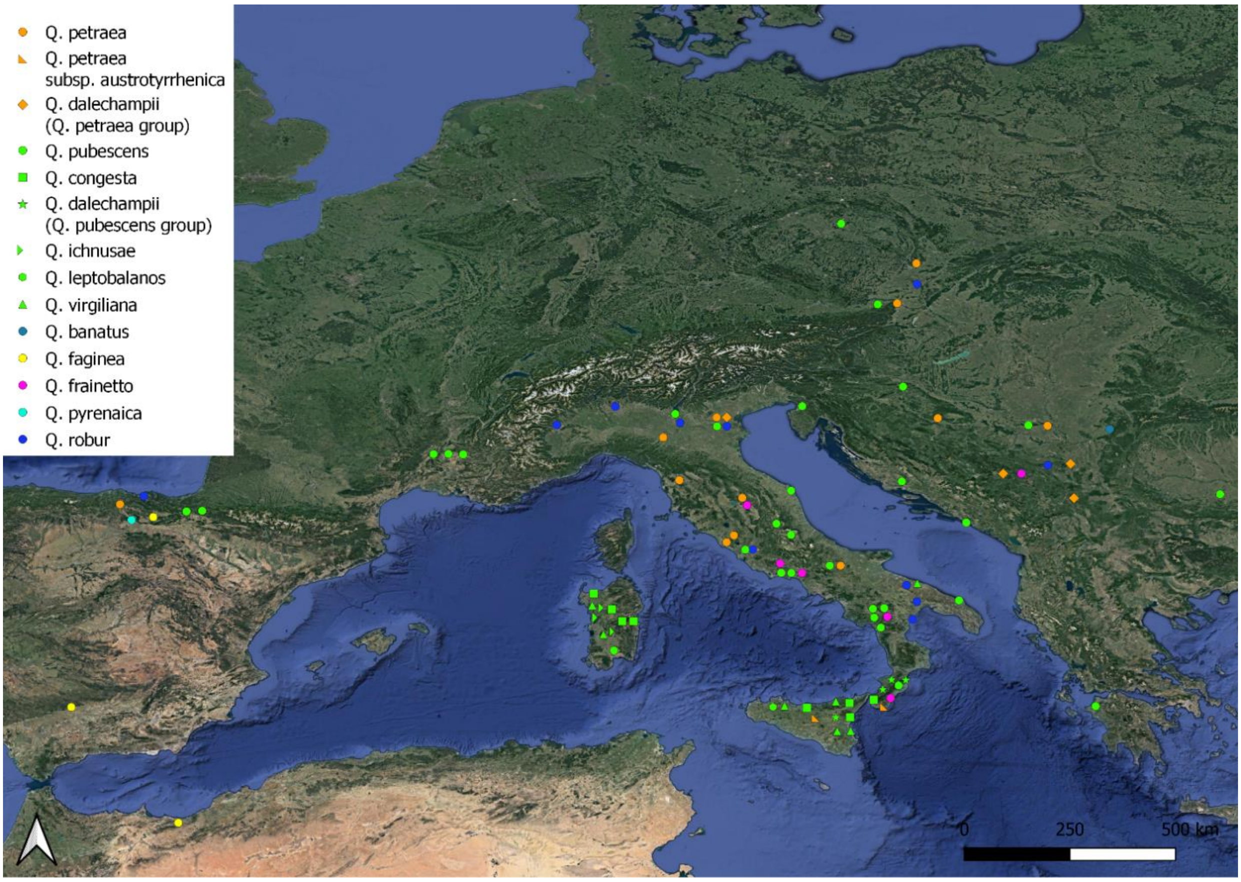


Figure 2.1 Distribution map of the *taxa* of the *Quercus* genus sampled

The populations selection was performed following a biogeographic criterion, so the patterns of geographic distribution of plants and the factors that determine these patterns, such as, for example, altitude, light, soil, pH, were considered during samples collection. In addition, mostly for the Italian populations, the sampling was carried out considering the forest communities described in literature. Both natural areas and protected ones as Natural/Regional/National Parks or Biogenetic Nature Reserves were chosen for the sampling. Moreover, some species were collected, when possible, in the *locus classicus*, namely the locality from which a *taxon* was first described (e.g., *Quercus banatus* from Oravița, Romania; *Q. congesta* from Mount Etna, Sicily Island; *Q. dalechampii* from Aspromonte, Calabria (southern Italy); *Q. frainetto* from Aspromonte, Calabria (southern Italy); *Q. ichnusae* from Senis, Sardinia Island; *Q. leptobalanos* from Ficuzza Wood, Sicily Island; *Q. petraea* subsp. *austrotyrrhenica* from Pomieri Wood, Sicily Island).

2.2 Plant material

The sampling design included 1987 individuals (trees) belonging to 93 populations (63 of which are Italian) representing 13 *taxa* (*Quercus banatus* P. Kucera, *Q. congesta* C. Presl, *Q. dalechampii* Ten. (referred in the map of Fig. 2.1 as *Q. dalechampii* (*Q. pubescens* group) or *Q. dalechampii* (*Q. petraea* group) according to the geographical provenance, see the introduction for more details), *Q. faginea* Lam., *Q. frainetto* Ten., *Q. ichnusae* Mossa, Bacch. & Brullo, *Q. leptobalanos* Guss., *Q. petraea* (Matt.) Liebl., *Q. petraea* subsp. *austrotyrrhenica* Brullo, Guarino & Siracusa, *Q. pubescens* Willd., *Q. pyrenaica* Willd., *Q. robur* L., *Q. virgiliana* (Ten.) Ten. belonging to the section *Quercus* of the *Quercus* genus. The sampling was performed when trees were in leaf to maximise the assignment to the different species.

In order to avoid capturing family structures, individuals were chosen to be at least 30 m apart from each other. An average of 25 individuals was sampled from each population, with the exception of the populations from the Biogenetic Nature Reserves, where few samples were collected. Plant material (including twigs, leaves, buds, flowers, fruits) collected from each sample was preserved as herbarium voucher in the herbarium of the University of Molise (IS) (Thiers 2015). In addition, three to ten leaves from each tree were dried in silica gel for genetic analyses.

2.3 Chloroplast genome analyses

2.3.1 Plant material for the chloroplast genome analyses

For the chloroplast (cp) genome analyses, a total of 270 individuals (3 trees for each population) belonging to 90 populations of the *Quercus* genus were selected and investigated attempting to cover the distributions of all the *taxa* in the sampled area (Fig. 2.2; Supplementary File 2). All the chloroplast genome analyses were carried out in the “Molecular Ecology of Forest Trees” laboratory of the University of Tuscia (Viterbo, Italy).

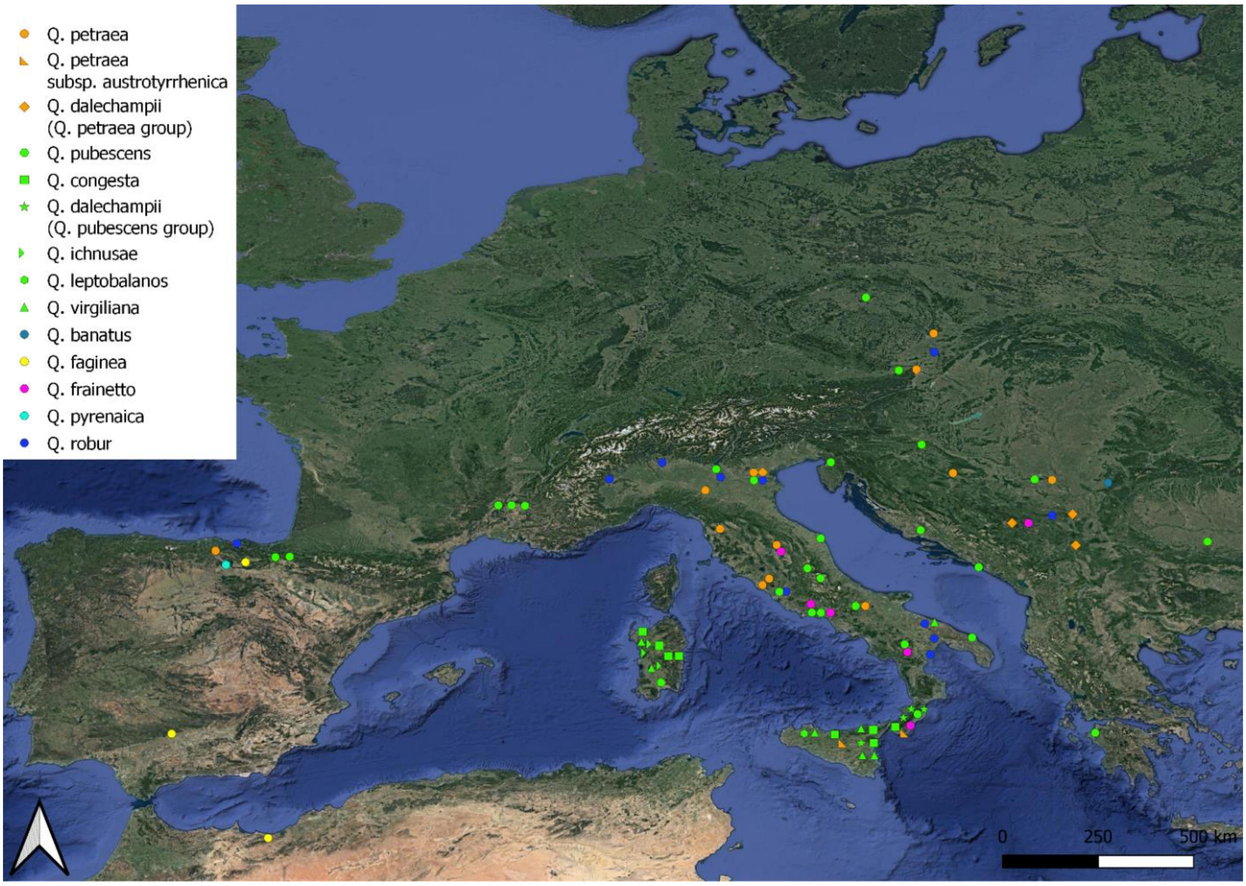


Figure 2.2 Distribution map of the *taxa* of the *Quercus* genus analysed by means of the chloroplast genome

2.3.2 DNA extraction, amplification, and sequencing

Genomic DNA was extracted from dried leaves (about 50 mg per tree) of 270 samples, with the NucleoSpin™ Plant II Kits (Macherey-Nagel), following the manufacturer's instructions.

NucleoSpin® Plant II kits allow the isolation of genomic DNA from plant tissue through two optimized lysis buffer systems based on the established CTAB and SDS methods. The plant samples were first homogenized by mechanical treatment. Then the DNA was extracted with Lysis Buffers PL1 containing chaotropic salts, denaturing agents, and detergents. Crude lysates were cleared by centrifugation and filtration using the NucleoSpin Filters provided with the kits in order to remove contaminations and residual cellular debris. The clear flow-through was then mixed with Binding Buffer PC to create conditions for optimal binding of DNA to the silica membrane. After that this mixture was loaded onto the spin column, contaminants were washed away using Wash Buffers PW1 and PW2. The genomic DNA was so eluted with low salt Elution Buffer PE (5 mM Tris/HCl, pH 8.5) or nuclease-free water and used for subsequent reactions (https://www.mn-net.com/).

Genomic DNA was then analysed by means of DNA sequence polymorphism at two plastid loci: the *trn*H-*psb*A intergenic spacer and a portion of the *trn*K-*mat*K region (3' intron and partial gene). These markers were selected because of their high number of accessible sequences on GenBank, and the variability displayed in previous studies (e.g., Manos et al. 2001; Okaura et al. 2007; Simeone et al. 2013, 2016).

The intergenic spacer *trn*H-*psb*A is the most widely utilized noncoding barcode in molecular phylogenetics following the coding *rbc*L and *mat*K sequences (CBOL PlantWorking Group 2009; Hollingsworth et al. 2011). Both two markers allow robust PCR amplifications from diverse plant *taxa* (Dong et al. 2012; Kress et al. 2005; Shaw et al. 2005; Shaw et al. 2007; Uncu and Uncu 2018). Primer sequences for *trn*H-*psb*A and *trn*K-*mat*K were obtained from Shaw et al. (2005) and Piredda et al. (2011), respectively (Table 2.1).

Table 2.1 Pairs of primers used for the amplification of the two marker fragments

Locus	Primer Name	Sequence
trnH-psbA	trnH-psbA_fw	5'-CGCGCATGGTGGATTCACAATCC-3'
	trnH-psbA_rev	3'-GTTATGCATGAACGTAATGCTC-5'
trnK-matK	matK_fw	5'-GTACTTGATGCGGGAAATGC -3'
	matK_rev	3'-CAATGATTGCAAATCCTTCTGA -5'

DNA sequences were then amplified in a total volume of 25 µl using PuReTaq Ready-To-Go PCR Beads (Cytiva) following the manufacturer’s instructions.

PuReTaq Ready-To-Go PCR Beads are pre-mixed, predispensed, single-dose reactions designed to performe standard PCR amplifications. The use of recombinant PuReTaq DNA polymerase and other high-purity reagents ensures reliable and robust performance in PCR amplifications and guarantees the lowest possible levels of contaminating prokaryotic and eukaryotic nucleic acids. The PuReTaq Ready-To-Go PCR Beads assure greater reproducibility between reactions, minimize pipetting steps, and reduce the potential for pipetting errors and contamination (https://www.cytivalifesciences.com/en/us).

Uniform PCR procedures were followed for all *taxa* and for the two plastid loci (*trn*H-*psb*A and *trn*K-*mat*K). The amplification reaction was carried out in an MJ Mini Bio Rad thermal cycler with the following profile: initial denaturation at 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 53 °C for 40 seconds, and elongation at 72 °C for 40 seconds, with a final extension step at 72 °C for 5 minutes.

PCR products were cleaned with Illustra$^{\text{TM}}$ GFX$^{\text{TM}}$ PCR DNA and Gel Band Purification Kit (GE Healthcare), following the manufacturer’s instructions.

The Kit allows rapid purification and concentration of DNA fragments with sizes ranging from 50 bp to 40 kb from PCR mixtures or agarose gel bands. The binding capacity is approximately 25 µg/column. The kit combines a chaotropic buffer with a glass-fiber matrix supported in a spin column for the purification of DNA from both solution and agarose gel. DNA purity is very high, and the 99.5% of contaminants are removed (https://www.cytivalifesciences.com/en/us).

Standardized aliquots of PCR products purified were then sent to Macrogen Europe (https://www.macrogen-europe.com/) for sequencing. For all samples, forward DNA strands sequencing was performed; additionally, bi-directional sequencing was performed if the results were unclear.

2.3.3 Data analysis

Electropherograms so obtained were edited with CHROMAS 2.6.6 (http://www.technelysium.com.au) and checked visually.

Multiple alignments were realized using MEGA 10.2.6 (Tamura et al. 2011). In addition, with the same software, pairwise uncorrected p-genetic distances (p), namely the proportion of observed differences between the two aligned genomes (Criscuolo 2020), were calculated.

DNAsp 6.12.03 (Librado and Rozas 2009) was used to generate haplotype lists and to analyse and calculate the number of haplotypes (H), the haplotype diversity (Hd), also known as gene diversity, that is the probability that two randomly sampled alleles are different (Nei 1978), and to evaluate the number of Parsimony Informative Characters (PICs), namely sites containing at least two types of nucleotides, in which at least two of them occur with a minimum frequency of two (Tamura et al. 2011).

These parameters were calculated for the sequences of the samples of *Quercus* investigated in this study. In addition, sequences from sections *Cerris* (Simeone et al. 2018) and *Ilex* (Simeone et al. 2016), as well as from West Eurasian *Quercus* (Simeone et al. 2013) and East Eurasian *Quercus* (Yang et al. 2020) were also studied in order to make comparisons. All these sequences are deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) (accession numbers are reported in Supplementary File 3).

NETWORK 10.2.0.0 (https://www.fluxus-engineering.com/) was used to make inferences about biogeography and history of the populations and to reconstruct genealogical relations between cpDNA haplotypes. The median-joining (MJ) algorithm was selected, and it was invoked with default parameters (equal weight of transversion/transition), in order to handle large datasets and multistate characters. It is an exceptionally fast method that can analyse thousands of haplotypes in a reasonable amount of time.

MJ method begins by combining the minimum spanning trees within a single network. The minimum spanning tree connects all given sequence types without creating any cycles or inferring additional (ancestral) nodes, resulting in a total length (i.e., the sum of distances between linked sequence types) that is as short as possible. With a parsimony criterion, median vectors, that can be biologically interpreted as possibly extant unsampled sequences or extinct ancestral sequences, are added to the network (Posada and Crandall 2001).

Median-Joining network was generated for the samples of *Quercus* analysed in this study. Furthermore, in order to reconstruct genealogical relations among cpDNA haplotypes of the samples belonging to the different sections of the subgenus *Quercus* (*Quercus*, *Lobatae*, *Protobalanus*, *Virentes*, *Ponticae*), with the addition of an outgroup represented by a sequence of a sample of the monotypic genus *Notholithocarpus* (closely related to *Quercus*, *Castanea*, and *Castanopsis* (Manos et al. 2008)), a Median-Joining network with star contraction, as a pre-processing option, was created. The star contraction option allows to simplify the network and to recognize population expansion events, and it can identify clusters among the haplotypes and create a contraction that looks like a star (Kong et al. 2016). Accession numbers of the sequences included in the final network are reported in Supplementary File 3.

Subsequently, a planar phylogenetic network was generated using Neighbour-Net (NNet) algorithm implemented in SPLITSTREE 4.18.3 (Huson and Bryant 2006). Given an alignment of sequences, a distance matrix or a set of trees, this program allows to generate a phylogenetic tree or network using methods such as split decomposition, neighbor-net, consensus network, super networks methods or other methods for calculating hybridization or simple recombination networks. In this study, the parameters of split transformation, distance transformation, character transformation, variance, and bootstrap replicates were set to equal angle, neighbor net, uncorrected p distance, ordinary least squares, and 1000, respectively. Other parameters followed default settings. More in detail, the equal angle algorithm guarantees that no two edges intersect (Kloepper and Huson 2008), while the neighbor-net algorithm is a method for constructing a collection of weighted splits which is then converted to a graphical representation, called a splits graph, using the drawing algorithms implemented in SplitsTree (Huson and Bryant 2006).

Splitstree graph was generated combining the sequences of the samples of *Quercus* analysed in this study, with the sequences of the subgenus *Quercus* belonging to the different sections, and an outgroup set represented by samples of the subgenus *Cerris* (represented by members of sections *Cyclobalanopsis*, *Cerris* and *Ilex*; this latter including all the currently identified main lineages: East Asian, WAHEA and Euro-Med according to Simeone et al. 2016). All these sequences are deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) (accession numbers are reported in Supplementary File 3).

Moreover, on the same dataset (Supplementary File 3), phylogenetic tree inference and bootstrap analyses were performed under maximum likelihood with RAxML v. 8.2.11 (Stamatakis 2014). The GTR+CAT approximation model and the ‘extended majority-rule consensus’ criterion as bootstrapping option (Pattengale et al. 2010), with 1000 bootstrap (BS) pseudoreplicates were used to assess branch support (BS). The CAT model is a computational work-around for the widely used General Time Reversible model of nucleotide substitution under the Gamma model of rate heterogeneity (GTR-Γ). The RAxML tree was rooted between subgenus *Cerris* and subgenus *Quercus*, following Zhou et al. (2022) and imported in iTOL (https://itol.embl.de/; Letunic and Bork 2021) for visualization and labelling.

2.3.4 Molecular markers

2.3.4.1 trnH-psbA

*Trn*H-*psb*A is a non-coding intergenic spacer and is among the most variable plant barcodes (Dong et al. 2012; Shaw et al. 2007). It ranges from 198 to 1077 bp, with an average length of 465 bp. The ends of this spacer, roughly 75 bp from either gene, are relatively conserved compared to its middle portion, which is highly indel prone (Aldrich et al. 1988) and contains several poly-A/T runs (Shaw et al. 2005). According to Kress et al. (2005), Shaw et al. (2007) and Whitlock et al. (2010), the *trn*H-*psb*A region shows many of the features deemed desirable in a barcode, in many plant lineages. In fact, *trn*H-*psb*A is a useful marker for different reasons:

-it is among the most variable (in terms of percentage variability)

-since it is a relatively short region across angiosperms, effective PCR amplification and sequencing from both high molecular weight and degraded DNA is possible

-one primer pair is likely to amplify nearly all angiosperm *taxa* since published primers seem to be "universal" (Shaw et al. 2007).

On the other side, given its short length, this region might not produce enough PICs to distinguish between closely related species (apart from the fact that closely related plants will be difficult to "barcode", because it has been shown that recent histories of hybridization can homogenize or even uncouple plastid genome phylogenies from species phylogenies) (Shaw and Small 2005). So, within some groups, *trn*H-*psb*A is not sufficiently variable to distinguish among closely related species (Sass et al 2007; Spooner 2009) and in others intraspecific variation is high (Edwards et al. 2008).

2.3.4.2 *trnK-matK*

Numerous evolutionary analyses have demonstrated the high degree of variability of the locus *mat*K (Shaw et al. 2005), considered one of the barcoding regions with the greatest universality through a variety of published works (Piredda et al. 2011). The marker *trn*K-*mat*K consists of the *mat*K gene and two flanking introns. The *mat*K coding region has a length of 1500-1600 bp in most angiosperms and is located within domain V of the *trn*K UUU group II intron (Neuhaus and Link 1987). The *mat*K open reading frame (ORF) is encoded by the *trn*K intron (Neuhaus and Link 1987), and it can be used as a marker to construct plant phylogenies because the ORF evolves rapidly, about three times faster than *rbc*L (Soltis et al. 1996) and is ubiquitous in plants (Hilu and Liang 1997; Kelchner 2002; Johnson and Soltis 1995). This ORF has been suggested to code for a maturase, based on structural similarities, and *mat*K is the only maturase found in higher plant plastids (Neuhaus and Link 1987). Likewise, the *trn*K intron is of interest because it represents an unusual form of a group II intron (Hausner et al. 2006).

The position of the two plastid loci studied (*trn*H-*psb*A and *trn*K-*mat*K) is shown in an example of the plastid genome, obtained from chloroplast genomes belonging to a total of 24 species of the *Quercus* genus (Pang et al. 2019) (Fig. 2.3).

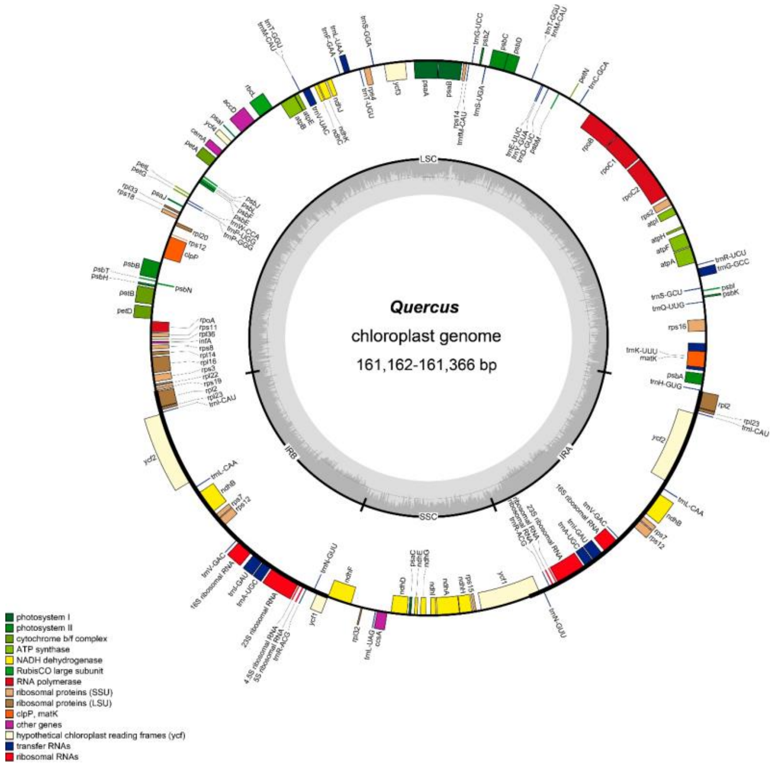


Figure 2.3 Chloroplast genome map of *Quercus*. Genes drawn inside the circle are transcribed clockwise, while those drawn outside are transcribed counterclockwise. Different colours are used to show genes in different functional groups. The extent of the inverted repeats (IRa and IRb), which separate the genomes into small single-copy (SSC) and large single-copy (LSC) regions, is indicated by dark bold lines (image from Pang et al. 2019)

2.4 Nuclear microsatellite markers analyses

2.4.1 Plant material for the nuclear microsatellite markers analyses

For the microsatellite (simple sequence repeat (SSR)) markers analyses, a total of 1072 individuals (20 trees for each population, except for 9 populations with less samples) belonging to 58 populations of the *Quercus* genus were selected and investigated (Fig. 2.4; Supplementary File 4). All the SSR analyses were carried out in the laboratories of the “Department of Forest Genetics and Forest Tree Breeding” of the University of Göttingen (Göttingen, Germany).

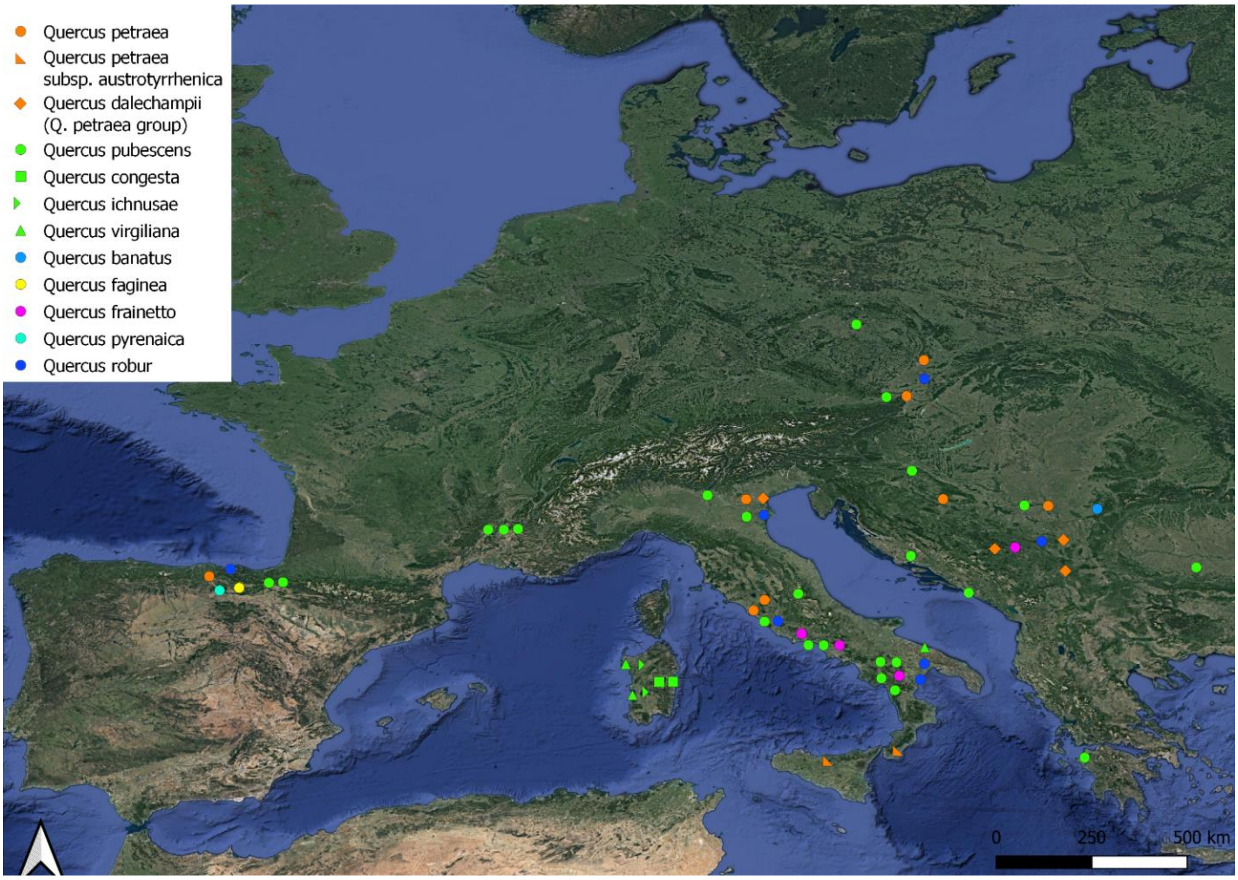


Figure 2.4 Distribution map of the *taxa* of the *Quercus* genus analysed by means of SSRs

2.4.2 DNA extraction, amplification, and sequencing

Genomic DNA was extracted from dried leaves (about 20 mg per tree) of 1072 samples, with two different DNA extraction Kits, the Qiagen DNeasy™ Plant Kit (Qiagen, Hilden, Germany) (samples 1-192; 673-1072) and the EchoLUTION Plant DNA Kit BioEcho (Cologne, Germany) (samples 193-672), following the manufacturer’s instructions.

With the Qiagen DNeasy™ Plant Kit, the samples were first mechanically disrupted and then chemically lysed. RNA was removed by RNase digestion during lysis. Centrifugation allowed to remove cell debris, precipitated proteins, and polysaccharides. The lysates were loaded onto the DNAeasy Plant 96-well plate. During a brief spin, DNA selectively binds to the silica membrane

while contaminants pass through. One wash step was used to remove remaining contaminants and enzyme inhibitors. Pure DNA was then eluted in water (https://www.qiagen.com/de-us).
With the EchoLUTION Plant DNA Kit BioEcho, first the Bead-beating Master Mix was added to the samples, and after centrifugation, Lysis Master Mix was added in the wells. The samples were then incubated with maximum agitation. After purification and elution, DNA was ready for use (https://www.bioecho.com/Products/DNA-Extraction/).

The samples were genotyped at twelve Expressed Sequence Tag-Simple Sequence Repeats (EST-SSRs), that were selected, based on the level of polymorphism and according to other studies on European white oaks (Lepais et al. 2009; Guichoux et al. 2011a, b; Neophytou et al. 2010; Curtu et al. 2015; Antonecchia et al. 2015; Di Pietro et al. 2021). The set of EST-SSR markers includes: PIE239, PIE227, PIE223, PIE215, PIE020, PIE152, PIE243, PIE242, PIE267, PIE102, PIE258, PIE271 (Durand et al. 2010) (Table 2.2).
EST-SSR markers derive from expressed, potentially functional regions of the genome, and because of their lower mutation rates, they are usually less polymorphic than genomic SSRs (Ellis and Burke 2007; Varshney et al. 2005). Nevertheless, EST-SSRs have relative advantage over genomic SSR because they are quickly obtained by electronic search, and they reflect the genetic diversity inside or adjacent to the genes (Varshney et al. 2005; Parthiban et al. 2018).
Three different multiplex reactions were used to combine the primers pairs for nuclear SSRs: Mu1 (PIE239, PIE227, PIE223, PIE215); Mu2 (PIE020, PIE152, PIE243, PIE242); Mu3 (PIE267, PIE102, PIE258, PIE271).
For each single primer pair, PCR reactions were conducted in a 13 µl volume containing 1 µl of genomic DNA (about 0.6 ng/µl), 1.5 µl of PCR buffer (containing 0.8 M Tris–HCl and 0.2 M $(NH_4)2SO_4$), 1.5 µl of $MgCl_2$ (25 mM), 1 µl of each dNTP (2.5 mM), 0.2 µl of HOT FIREPol® Taq Polymerase from Solis BioDyne (Tartu, Estonia), 6.7-7.5 µl of ddH_2O (quantity varied according to the quantity of primers), 0.15-0.55 µl of primer (forward, 5 pM/µl) and 0.15-0.55 µl of primer (reverse, 5 pM/µl). More in detail, for primer forward and reverse were used: 0.15 µl for PIE020, PIE243, PIE267; 0.2 µl for PIE239, PIE227, PIE223; 0.4 µl for PIE215, PIE102, PIE258, PIE271; 0.5 µl for PIE242; 0.55 µl for PIE152.
Uniform PCR procedures were followed for all *taxa* and for the twelve markers. A touchdown PCR program was selected and performed in a Biometra Thermocycler TOptical Gradient 96 (Biometra, Goettingen, Germany). Touchdown profile, useful if the optimal annealing temperature is not known, incrementally decreases the annealing temperature in early cycles to maximize the yield of specific products. The PCR protocol started with denaturation at 95 °C for 15 minutes, followed by 10 cycles of 1 minute denaturation at 94 °C, annealing at 60 °C (−1 °C per cycle) for 1 minute, and extension at 72 °C for 1 minute. The second step consisted of 25 cycles of 1 min denaturation at 94 °C, 1 min annealing at 50 °C and 1 min extension at 72 °C, followed by a final extension step of 72 °C for 20 minutes.

To determine the SSR sizes, capillary electrophoresis was run on an ABI3130XL genetic analyser (Applied Biosystems, USA). The GS500 size standard was used. To analyse fragment sizes and for genotyping, Software GeneMapper v4.0 (Applied Biosystems) was used. Allele values set by the software were checked for consistency and, if necessary, corrected manually. In order to rule out systematic genotyping errors, analyses were repeated for dubious results.

Allele calling and binning, the process that converts raw allele lengths into allele classes with a size expressed by an integer (Idury and Cardon 1997), was performed by visual assignment.

Table 2.2 Observed size range, motif types, primer sequences, and dye type of the SSRs analysed

	Locus	Observed size range (bp)	Motif type	Forward primer (5′–3′)	Reverse primer (3′–5′)	Dye type
Mu1	**PIE239**	79-110	$(AT)_{12}$	CAA CAA ATG GCT CAA CAG TG	CCC ATT TGG TAG CAA AGA GTC	6-FAM
	PIE227	150-179	$(TGG)_{8}$	ACC ATG ATC TGG GAA GCA AC	AAG GGC TTG GTT GGG TTA GT	6-FAM
	PIE223	190-237	$(GGT)_{8}$	AGA AGC CCA ACA CGG CTA C	AGC AAA ACA CAA ACG CAC AA	6-FAM
	PIE215	187-224	$(GAG)_{6}$	ACG AAA TGG AGC TGT TGA CC	TCT CCT TCT CTT CTG CCA TGA	HEX
Mu2	**PIE020**	97-122	$(TA)_{12}$	GCA GAG GCT CTT CTA AAT ACA GAA CT	GGG AGG TTT CTG GGA GAG AT	6-FAM
	PIE152	225-265	$(AG)_{11}$	TGT ACC TCT TTC CTC TCT CTA AAA CT	GAA TTT CTA AAC CAC TAG CAT TGA C	HEX
	PIE243	200-235	$(AG)_{15}$	GGG GTC AGT AGG CAA GTC TTC	GAG CTG CAT ATT TTC CTT AGT CAG	6-FAM
	PIE242	100-130	$(TA)_{10}$	GGA GGG AAA AGA ACA ATG C	TTG CAA TCC TCC AAA TTT AATG	HEX
Mu3	**PIE267**	86-110	$(AG)_{11}$	CCA ACC ATC AAG GCC ATT AC	GTG CGA ACA GAT CCC TTG TC	6-FAM
	PIE102	132-177	$(AG)_{12}$	ACC TTC CAT GCT CAA AGA TG	GCT GGT GAT ACA AGT GTT TGG	HEX
	PIE258	128-164	$(TC)_{13}$	TCT CGA TCT CAA AAC AAA ACCA	TTT GAT TTG TTT AAG GAA AAT TGG A	6-FAM
	PIE271	182-221	$(TC)_{11}$	CAC ACT CAC CAA CCC TAC CC	GTG CGG TTG TAG ACG GAG AT	HEX

2.4.3 Data analysis

Genetic analyses of samples were performed on different set of data. As two markers, PIE239 and PIE258, did not amplify correctly, they were not considered in all the analyses. In addition, because unbalanced data in the populations affected the main genetic statistics, seven populations with a number of samples lower than ten (IT82, IT72, IT66, BG03, IT21, IT62, RS39) were excluded from the main analyses, while they were used for genetic structure analyses and phylogenetic analysis, where the number of samples did not affect the results.

2.4.3.1 Genetic Structure Analysis by Bayesian clustering

STRUCTURE 2.3.4 (Pritchard et al. 2000) was used, on the combined SSRs data of the samples, to assess patterns of the genetic structure in the 58 studied populations.

A Bayesian iterative method (Markov Chain Monte Carlo (MCMC) estimation) is used in this software to analyse differences in the distribution of genetic variants among populations by placing samples into groups whose members share similar patterns of variation. Furthermore, STRUCTURE both identifies populations from the data and assigns individuals to that population representing the best fit for the variation patterns found (Porras-Hurtado et al. 2013).

An admixture model (Alpha, α) without prior information on the geographical location of samples or the taxonomical classification and an allele frequency model (Lambda, λ) were run. Twenty replications were performed for each value of clusters K, considering values of K from one to twelve (the putative number of *taxa* in the input dataset), with a burn-in length of 50000 and a run length of 100000 iterations.

According to Markov Chain Monte Carlo (MCMC) estimation, after randomly allocating samples to a predetermined number of groups, variant frequencies are estimated in each group, and individuals are subsequently re-assigned based on those frequencies. The burn-in process leads to a progressive convergence toward reliable allele frequency estimates in each population and membership probabilities of individuals to a population (Porras-Hurtado et al. 2013).

The membership coefficient (expressed by the Q value) was used to evaluate the belonging of each sample to a cluster and it was set a threshold of $Q \geq 0.90$ to identify samples as pure species.

According to Wang (2017) and Di Pietro et al. (2021), due to the unbalanced number of samples in the 58 populations, the Alpha value was set to $1/N$ (where $N = 12$, namely the putative number of *taxa* in the input dataset). In addition, the degree of admixture "Alpha", as well as "Lambda" value were set to be inferred for each population.

STRUCTURE results were then processed in STRUCTURE HARVESTER (Earl and von Holdt 2012). This program allows to assess and visualize likelihood values across multiple values of K and hundreds of iterations for easier detection of the number of genetic clusters that best fit the data (Earl and von Holdt 2012).

More in detail, an estimate of the posterior probability of the data for a given K, $\Pr(X|K)$ is used as the model choice criterion to detect the true K (Pritchard et al. 2000).

This value, called 'Ln P(D)' in STRUCTURE output, is obtained by first computing the log likelihood of the data at each step of the MCMC. Then the average of these values is computed, and half their variance is subtracted to the mean. True number of groups (K) is often identified using the maximal value of $L(K)$ returned by STRUCTURE (Evanno et al. 2005).

Graphic representation and summary of STRUCTURE results were obtained with CLUMPAK software (Kopelman et al. 2015).

2.4.3.2 Phylogenetic Analysis

The hierarchical structure of the sampled populations was then analysed with Populations software v. 1.2.32 (Langella 1999). Samples with $Q \geq 0.90$ for a different cluster than the pre-defined population were removed from the analysis. A phylogenetic tree of populations was built with the Neighbour-Joining (NJ) method and the chord genetic distance of Cavalli-Sforza and Edwards (1967). Bootstrapping was carried out with 999 replicates over loci, using the grouped population option. MEGA 10.2.6 (Tamura et al. 2011) was afterwards used to display the tree.

2.4.3.3 Genetic diversity and differentiation

Genetic diversity and differentiation within and among populations were estimated in order to evaluate genetic variation.

Cervus 3.0.7 (Marshall et al. 1998) was used to calculate the number of individuals analysed (N), the number of alleles (K), and polymorphic information content (PIC) per locus.

Polymorphic Information Content (PIC) is one of the indicators of marker quality in genetic studies. It describes the capacity to detect the polymorphism among individuals of a population, and the higher that capacity, the greater its value (Serrote et al. 2020). PIC values for codominant markers range from 0 (monomorphic) to 1 (very highly informative, with several alleles of equal frequency). Markers with PIC values greater than 0.5 are regarded to be very informative, values between 0.25 and 0.50 are slightly informative, and values lower than 0.25 are not very informative (Botstein et al. 1980).

The mean number of alleles (N_a) per population and number of private alleles (N_p), namely the alleles found only in a single population (Petit et al. 1998), were then calculated with GenAlEx software v. 6.5 (Peakall and Smouse 2012). While, missing genotypes, observed (H_o) and expected (H_e) heterozygosity per population were assessed in SPAGeDI-1.3.d (Hardy and Vekemans 2002).

Heterozygosity (also called gene diversity) is the main parameter to describe genetic variation at single locus or at different loci, assuming that alleles are randomly chosen in different populations (Nei et al. 1983). Specifically, the observed heterozygosity is assessed from individual genotypes (H_o = Direct count of heterozygote / Total number of individuals surveyed) and depends on both the amount of genetic variation in the population and the level of inbreeding (Dorji and Daugjinda 2014; Ritland 1996), while expected heterozygosity (H_e) for the population is based on the allele frequency of the sampled individuals given that the population is in Hardy Weinberg Equilibrium (HWE) (Schmidt et al. 2020). The Hardy Weinberg principle states that genotype frequencies can be predicted from gene/allele frequencies and remain constant over generations in random-mating populations under the simple assumptions of no mutation, selection, or migration (Garnier-Géré and Chikhi 2013). Inbreeding and/or genotyping problems can lead to a deviation of population from HWE. When the observed heterozygosity is higher than expected heterozygosity ($H_o>H_e$) then it might be the result of two previously isolated populations mixing, when the population is likely to be in random mating then $H_e=H_o$ (Mburu and Hanotte 2005), while the presence of inbreeding can be observed when expected heterozygosity is significantly higher than observed heterozygosity ($H_o<H_e$) (Schmidt et al. 2020).

HP-Rare software v. June-6-2006 (Kalinowski 2005) was used to calculate allelic richness (A_r) after rarefaction for each population. Because large genetic samples are expected to have more alleles than small samples, the sample size heavily affects the number of distinct alleles and the number of private alleles, so they can be difficult to interpret when sample sizes differ across populations (Szpiech et al. 2008).

Rarefaction is a statistical technique that allows to produce estimates that are comparable in different populations (Hurlbert 1971; Kalinowski 2004, 2005; Petit et al. 1998).
Unequal samples are cut off to the same standardized sample size, which must be smaller than or equal to the smallest sample size across populations, consequently the estimated allelic richness of a population is the number of distinct alleles expected in a random subsample of size *g* drawn from the population (Hurlbert 1971; Petit et al. 1998).
Rarefaction size set for the samples of this study was 28, corresponding to the minimum sample size, namely the minimum number of successful amplifications at one locus within a population.

SPAGeDI-1.5.d software (Hardy and Vekemans 2002) was also used to calculate Wright's fixation indices (Wright 1969), including inbreeding coefficient (F_{IS}) per population and fixation index (F_{ST}) among populations. Wright's fixation indices, or F-statistics (Table 2.3), are the parameters most widely used to describe population structure (Nagylaki 1997) and to analyse the degree of subpopulation division and breeding system when two gametes are drawn randomly from each subpopulation (Wright 1951). These indices include: Wright's fixation coefficient of an individual within a subpopulation (F_{IS}), fixation coefficient of an individual within the total population (F_{IT}), and fixation coefficient of a subpopulation within the total population (F_{ST}) (Dorji and Daugjinda 2014). F-statistics are usually defined for a two-level hierarchy, with individuals in subpopulations. From such a hierarchy, F_{IS} (where "I" stands for individual and "S" for subpopulation), also called inbreeding coefficient, denotes the degree of HWE departure in subpopulations due to genetic inbreeding, and it ranges from −1 (maximum outbreeding) to 1 (inbreeding) (Nassiry et al. 2009). F_{ST} (where "S" stands for subpopulation and "T" for total) quantifies the differences in allele frequencies among subpopulations (Goudet 2005). Because subpopulation division brings to heterozygotes deficiency, F_{ST} is always positive, and it ranges from 0 to 1 (Nassiry et al. 2009). So, when there is no subpopulation division, F_{ST} is 0 because there is no genetic variation among populations. F_{IT} (where "I" stands for individual and "T" for total) is rarely used to determine HWE deviation in the total population, and it ranges between −1 (maximum outbreeding) to 1 (inbreeding) (Nassiry et al. 2009).

Table 2.3 Fixation indices (F_{IS}, F_{ST}, F_{IT}). H_S = mean expected heterozygosity within random mating subpopulations. H_T = expected heterozygosity in random mating total population. H_I = mean observed heterozygosity per individual within subpopulations (Nassiry et al. 2009)

Fixation index	Formula
F_{IS}	$F_{IS} = \frac{(H_S - H_I)}{H_S}$
F_{ST}	$F_{ST} = \frac{(H_T - H_S)}{H_T}$
F_{IT}	$F_{IT} = \frac{(H_T - H_I)}{H_T}$

Occurrence of null alleles were subsequently checked using the software Microchecker 2.3.4 (Van Oosterhout et al. 2004).

A microsatellite null (non-amplifiable) allele is any allele at a microsatellite locus that consistently fails to amplify to detected levels via the polymerase chain reaction (PCR) (Brookfield 1996; Dakin and Avise 2004). Microsatellite null alleles are found to a varying degree across all *taxa*. They can be problematic as they may affect genetic differentiation measures and create false homozygotes, generating an overall significant homozygote excess (Carlsson 2008).

In fact, in the presence of substantial proportion of null alleles at a particular locus, the observed heterozygosity would be largely underestimated. Consequently, null alleles affect especially population parameter estimates, which are based on the proportion of heterozygotes (Chybicki and Burczyk 2009).

After having loaded each population separately and having entered the repeat motif for each of the loci in Microchecker, 1000 randomizations of alleles within each locus and population were applied for the tests. The frequency of null alleles per population and locus was then calculated by applying the expectation–maximization algorithm (Dempster et al. 1977) in FREENA (Chapuis and Estoup 2006).

2.4.3.4 Analysis of Molecular Variance

To further analyse the genetic structure of the studied populations, analysis of molecular variance (AMOVA) was performed with Arlequin v. 3.5.2.2 (Excoffier and Lischer 2010). The explained variance was estimated for three levels, represented by: populations (51 populations); individuals within populations (an average of 20 individuals per population) and individuals (1013 in total).

2.4.3.5 Principal Coordinates Analysis

Subsequently, a Principal Coordinates Analysis (PCoA) based on the covariance standardised method (using the tri distance matrix) was performed on the pairwise population matrix of F_{ST} values obtained with GenAlEx 6.5 (Peakall and Smouse 2012).

2.4.3.6 Comparison of geographical and genetic distance

To evaluate spatial processes driving population structure, Mantel tests with 9999 permutations were performed with GenAlEx software v. 6.5. Pairwise genetic and geographic distances were first calculated between all the populations. The relationship between the two distances was expressed by the correlation coefficient (R_{XY}), a numerical index that ranges from − 1 to 1, where positive correlation means that two groups are genetically more distant from one another the farther apart they are from one another, while a negative correlation would suggest the opposite (Pettenkofer 2020).

3 Results

3.1 Results of the chloroplast genome analyses

3.1.1 Haplotype diversity

In total, 270 samples, belonging to 90 populations (3 trees for each population) of the *Quercus* genus, were analysed (Supplementary File 5). Both two barcoding loci, *trn*H-*psb*A and *trn*K-*mat*K, were successfully amplified and sequenced across all samples. Consequently, the final dataset included 540 plastid DNA sequences (270 samples, two markers each). The aligned sequences were calculated to have total lengths of 521 and 626 bp in *trn*H-*psb*A and *trn*K-*mat*K, respectively. The multiple alignment of the two concatenated markers resulted in a matrix of 1147 bp in length. The main features and the diversity values of the two investigated markers are summarized in Table 3.1. As expected, the variation encountered was moderate to low, and *trn*H-*psb*A performed better than *trn*K-*mat*K across all the three sections of the *Quercus* genus investigated. The length of sequences for each of the two markers was comparable in all the groups and, although the number of species and samples was different, section *Ilex*, which was one of the less numerous in terms of samples, showed the highest values of diversity. The uncorrected p-distance ranged between 0.00 and 0.0128, with the highest values in the section *Ilex* considering *trn*H-*psb*A marker. The number of haplotypes ranged from 4 (section *Quercus* (East Asian members) and *Cerris*, *trn*K-*mat*K) to 29 (section *Ilex*, *trn*H-*psb*A) and the haplotype diversity from 0.2007 (section *Cerris*, *trn*K-*mat*K) to 0.9142 (section *Ilex*, *trn*K-*mat*K), while the number of Parsimony Informative Characters (PICs) ranged from 1 (section *Cerris, trn*K-*mat*K) to 10 (section *Ilex, trn*K-*mat*K).

Table 3.1 Main diversity values of the *trn*H-*psb*A and *trn*K-*mat*K marker regions in section *Quercus*, subdivided into the here investigated European dataset (1), the expanded GenBank dataset comprising all available West Eurasian (2), and the East Asian (3) members of section *Quercus*, compared with GenBank data retrieved from West Eurasian members of subgenus *Cerris* (sections *Cerris* and *Ilex*). T: number of species (only species *sensu latu* were considered); N: number of individuals; p: uncorrected p-distance range (min. – max.); H: number of haplotypes (gaps included); Hd: haplotype diversity; PICs: Parsimony Informative Characters; T+K: combined markers; L: Major lineages identified (* = with only *trn*H-*psb*A considered; ** only single haplotypes were separated by >1 mutation)

		trnH-psbA						**trnK-matK**						**T+K**
Subgenus	**Section**	**T**	**N**	***p***	**H**	**Hd**	**PICs**	**T**	**N**	***p***	**H**	**Hd**	**PICs**	**L**
Quercus	Quercus^1	7	270	0.0-0.0058	9	0.7713	4	7	270	0.0-0.0032	5	0.5557	2	1
	Quercus^2	15	425	0.0-0.0089	19	0.8639	5	15	425	0.0-0.0051	8	0.543	2	1
	Quercus^3	9	81	0.0-0.0119	13	0.7568	5	9	81	0.0-0.0047	4	0.208	3	n.d.**
Cerris	Ilex	4	124	0.0-0.0128	29	0.8881	7	4	124	0.0-0.0116	23	0.9142	10	3
	Cerris	12	207	0.0-0.0082	12	0.5148	5	10	47	0.0-0.0014	4	0.2007	1	2*

3.1.2 Haplotype classification

Site variation observed in the 270 analysed samples resulted in fourteen total haplotypes (H01-H14). Nine haplotypes (T01-T09) were observed considering sequences of *trn*H-*psb*A, and five haplotypes (K01-K05) were identified considering sequences of *trn*K-*mat*K. No species-specific haplotypes were detected, consequently different haplotypes were shared by several *taxa* of the *Quercus* genus (Table 3.2; Supplementary File 5).

Table 3.2 Haplotypes occurrence in the *taxa* of *Quercus* analysed

Taxon	H01	H02	H03	H04	H05	H06	H07	H08	H09	H10	H11	H12	H13	H14
Q. banatus	X													
Q. congesta			X			X	X	X	X					
Q. dalechampii (*Q. pubescens* group)		X	X				X	X						
Q. dalechampii (*Q. petraea* group)	X	X												
Q. faginea			X	X									X	X
Q. frainetto	X	X		X		X								
Q. ichnusae							X		X	X				
Q. leptobalanos						X								
Q. petraea	X	X	X	X										
Q. petraea subsp. *austrotyrrhenica*		X				X								
Q. pubescens	X	X	X	X	X	X						X		
Q. pyrenaica				X										
Q. robur	X	X	X	X		X								
Q. virgiliana		X	X			X	X		X		X			

Considering the total haplotypes, haplotypes H01-H04 were the most common across the area of study and the *taxa* analysed. Haplotype H01 was the most prevalent with a frequency of 26.67% in total. The second most frequent haplotype was the haplotype H02 with a frequency of 22.59%. Haplotype H03 and H04 followed with a frequency of 16.3% and 11.48%, respectively. The remaining haplotypes had lower frequencies. Six haplotypes were rare (H05; H10-H14) with frequencies less than 1.12% (Table 3.3).

Table 3.3 Relative haplotype (*trn*H-*psb*A and *trn*K-*mat*K) frequencies in the 270 samples of *Quercus* analysed (pro *taxon*). N_{ind} = number of individuals, N_{pop} = number of populations, N_{ind} (%) = frequency of the number of individuals (%), N_{pop} (%) = frequency of the number of populations (%)

Taxon	N_{ind} and N_{pop}		H01	H02	H03	H04	H05	H06	H07	H08	H09	H10	H11	H12	H13	H14
Q. banatus	N_{ind}=3	Nind(%)=1.11	100													
	N_{pop}=1	Npop(%)=1.11														
Q. congesta	Nind=24	Nind(%)=8.89			37.5			8.33	12.5	16.7	25					
	Npop=8	Npop(%)=8.89														
Q. dalechampii (*Q. pubescens* group)	Nind=12	Nind(%)=4.44		41.7	25				8.33	25						
	Npop=4	Npop(%)=4.44														
Q. dalechampii (*Q. petraea* group)	Nind=12	Nind(%)=4.44	25	75												
	Npop=4	Npop(%)=4.44														
Q. faginea	Nind=9	Nind(%)=3.33			11.1	66.7									11.1	11.1
	Npop=3	Npop(%)=3.33														
Q. frainetto	Nind=18	Nind(%)=6.67	16.7	50		16.7		16.7								
	Npop=6	Npop(%)=6.67														
Q. ichnusae	Nind=9	Nind(%)=3.33							55.6		33.3	11.1				
	Npop=3	Npop(%)=3.33														
Q. leptobalanos	Nind=3	Nind(%)=1.11						100								
	Npop=1	Npop(%)=1.11														
Q. petraea	Nind=36	Nind(%)=13.33	41.7	30.6	16.7	11.1										
	Npop=12	Npop(%)=13.33														
Q. petraea subsp. *austrotyrrhenica*	Nind=6	Nind(%)=2.22		50				50								
	Npop=2	Npop(%)=2.22														
Q. pubescens	Nind=81	Nind(%)=30	34.6	22.2	11.1	16.1	3.7	11.1						1.2		
	Npop=27	Npop(%)=30														
Q. pyrenaica	Nind=3	Nind(%)=1.11				100										
	Npop=1	Npop(%)=1.11														
Q. robur	Nind=33	Nind(%)=12.22	60.6	9.11	18.2	6.05		6.05								
	Npop=11	Npop(%)=12.22														
Q. virgiliana	Nind=21	Nind(%)=7.78		14.3	47.6			4.76	14.3		14.3		4.8			
	Npop=7	Npop(%)=7.78														
Frequence of haplotype (%)			26.67	22.59	16.30	11.48	1.11	8.52	4.44	2.59	4.45	0.37	0.37	0.37	0.37	0.37

3.1.3 Geographic distribution of the haplotypes

Most populations were fixed for one haplotype, although fourteen out of ninety populations showed two (BG03: *Quercus pubescens* from Kushin, Bulgaria; IT14: *Q. robur* from Insugherata, Italy; IT22: *Q. pubescens* from One, Italy; IT27: *Q. dalechampii* from Serre Sant'Angelo, Italy; ES41: *Q. robur* from Leioa, Spain; ES42: *Q. faginea* from Zaldiaran, Spain; IT50: *Q. ichnusae* from Monte Traessu, Sardinia, Italy; IT55: *Q. virgiliana* from Marineo, Italy; IT60: *Q. congesta* from Madonie, Italy; IT73: *Q. petraea* from Monte Vairano, Italy; IT77: *Q. pubescens* from Bosco del Compare, Italy; IT84: *Q. robur* from Groane Park, Italy; IT85: *Q. robur* from Bosco Fontana, Italy) or three (ES85: *Q. faginea* from Cordoba, Spain) different haplotypes. Furthermore, while it was not found a relation between species and haplotypes, a more significant and direct association was recognized with the geographic distribution (Fig. 3.1), mostly for some haplotypes. More specifically, haplotypes H01 and H02 were the most common and were found in central eastern Europe: the first one in Austria, Croatia, Czech Republic, France, Italy, Romania, and the second one in Bulgaria, Croatia, Greece, Italy, and Serbia.

Haplotype H03 was found in the Italian Peninsula, including Sicily and Sardinia Islands, and in Spain. Haplotype H04 showed a discontinuous distribution, and it was found across the Italian and Iberian Peninsulas, in Morocco and in Bulgaria. As this haplotype was found in a single Bulgarian sample, this sample was re-sequenced several times, with the same results each time.

Haplotype H05 was exclusive of Croatia. Haplotype H06 was distributed across all the Italian Peninsula, as well as in Sicily Island. Haplotypes H07, H08, and H09 were found only in Italy: in Sardinia and in Calabria, in Calabria and Sicily, and in Sardinia, respectively. Haplotypes H10, H11, H12, H13, H14 were exclusive of one single sample represented respectively by: IT5072 (*Quercus ichnusae* from Monte Traessu, Italy), IT5501 (*Q. virgiliana* from Marineo, Italy), IT7712 (*Q. pubescens* from Bosco del Compare, Italy), ES8502 and ES8503 (*Q. faginea* from Cordoba, Spain). Focusing on the Italian Peninsula, 11 plastid haplotypes (H01-H04; H06-H12) were detected in the 60 populations sampled (Supplementary File 6a). Furthermore, the geographic distribution of the plastid haplotypes considered for each *taxon* separately did not provide significant information about the species specificity of the haplotypes. As supporting information, the distribution of the haplotypes in the populations of *Quercus pubescens s.l.*, *Q. petraea s.l.*, *Q. robur*, and *Q. frainetto*, as well as the distribution of the rare plastid haplotypes found in the samples, is provided (Supplementary File 6b, 6c, 6d, 6e, 6f).

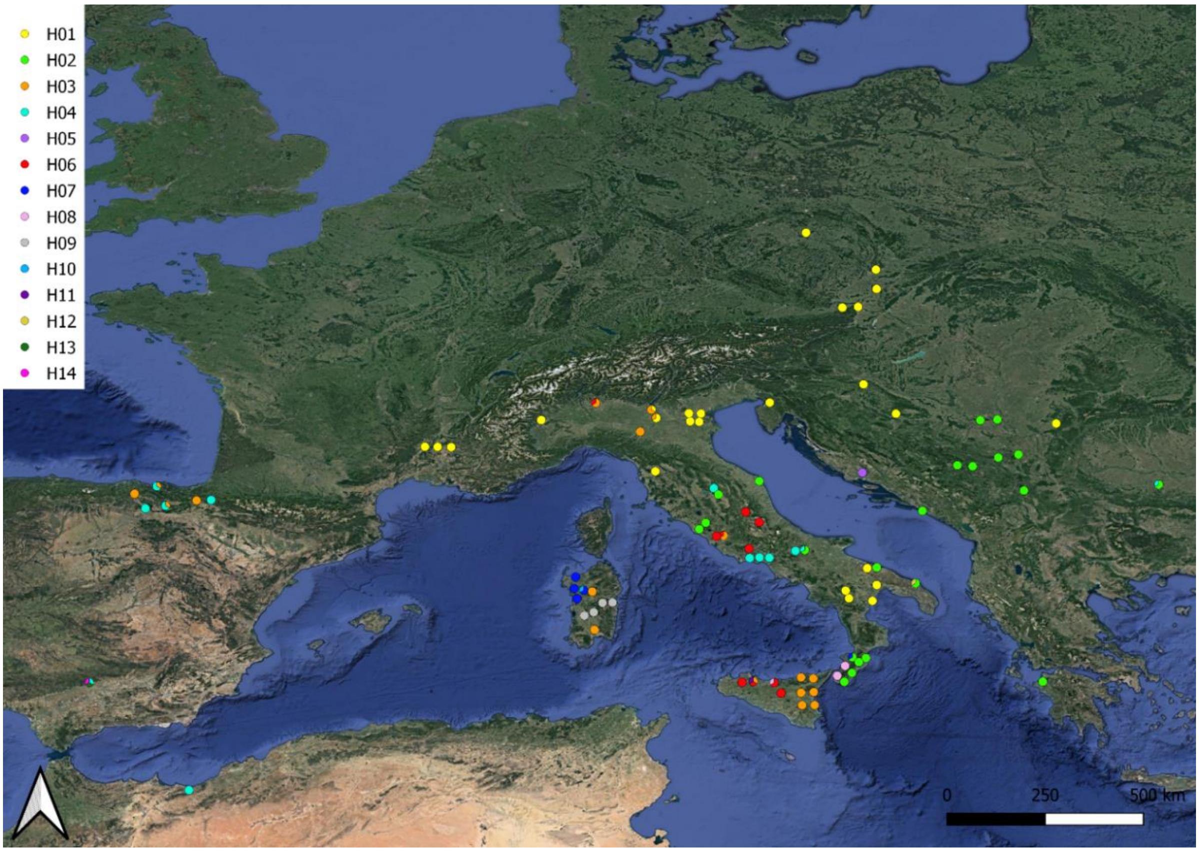


Figure 3.1 Geographic distribution of the plastid haplotypes in the populations of *Quercus* analysed

3.1.4 Genealogical relations among haplotypes

Genealogical relations among the fourteen haplotypes (H01-H14), based on variability of the two combined plastid loci (*trn*H-*psb*A and *trn*K-*mat*K) analysed in 270 individuals, are shown in the median joining network (Fig. 3.2). One or two mutations differentiated the fourteen haplotypes, providing evidence that the populations and the *taxa* of section *Quercus* diverged rather recently. The central haplotypes in the network were the most prevalent and spread haplotypes across the populations sampled, supporting the hypothesis that these belonged to the ancestral populations. Although clusters of haplotypes cannot be identified, because of the low rate of mutations, two major groups can be assumed. The first includes haplotypes H02, H01, H05 spread through Italian Peninsula, Austria, Czech Republic, France, and Balkan Peninsula, and the second (H03, H04, H06, H08) is represented by the haplotypes from Italian (Islands included) and Iberian Peninsulas, and by the Bulgarian sample with haplotype H04.

Furthermore, the presence of one hypothetical node that links the central haplotypes with the Spanish haplotypes H13 and H14 and with the Italian haplotypes H07 and H10 suggests that the inclusion of additional loci, which could improve the resolution, would help in resolving the genealogy. Haplotypes networks were also produced for the two plastid regions separately (Supplementary File 7a, 7b).

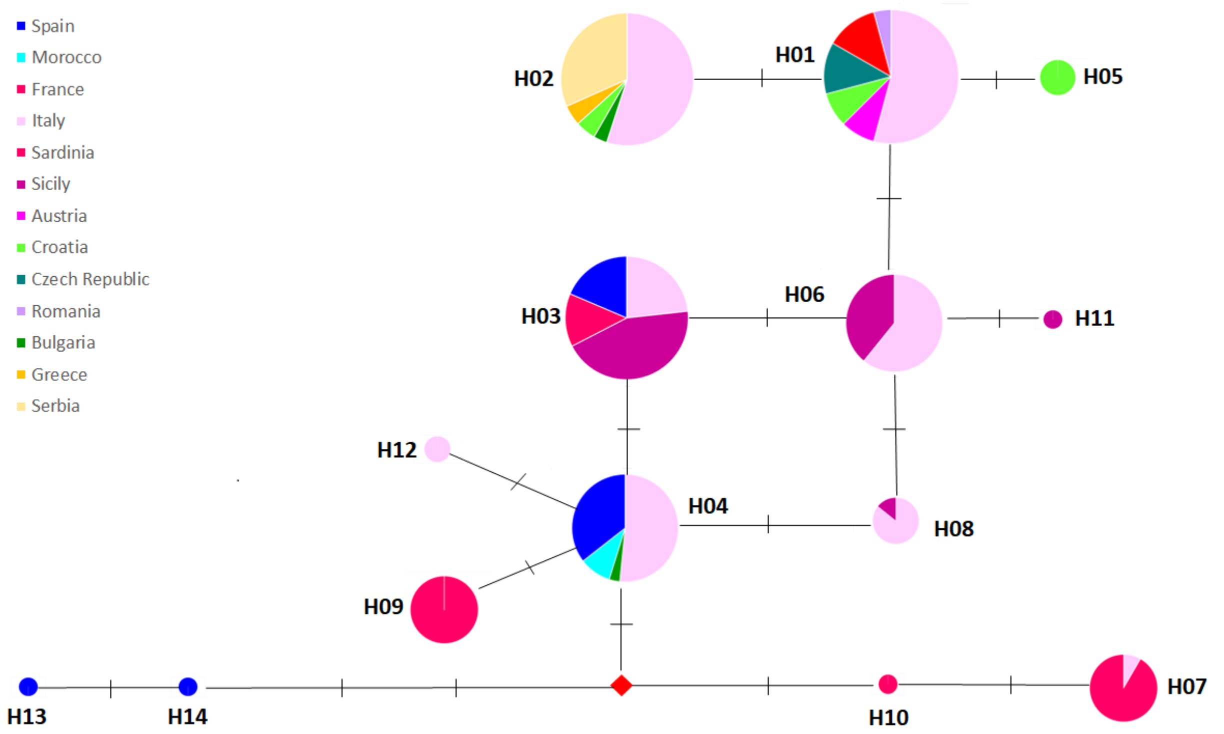


Figure 3.2 Median-joining network based on the two plastid loci (*trn*H-*psb*A and *trn*K-*mat*K) of the sequences of the 270 samples of *Quercus* analysed. Each sequenced haplotype is represented by a circle, the size of which is proportional to its overall frequency in the dataset (Supplementary File 5). The number of mutational changes between two distinct haplotypes is shown by the lines on the branches. The red diamond represents the median vector. Haplotype colours correspond to the sampling localities

More evident and consistent is the separation in clusters observed in the median joining network built for the cpDNA sequences belonging to different sections of subgenus *Quercus* (*Quercus*, *Ponticae*, *Virentes*, *Lobatae*, *Protobalanus*) with the addition of one sample of *Notholithocarpus densiflorus* as outgroup (Fig. 3.3). Separated clusters, attributable to the main sections and to the outgroup, can be indeed easily identified and recognized. The main differentiation appears between North American and Eurasian sections in the complex. The highly differentiated plastomes inside North American sections are highlighted by the high number of mutational changes between different haplotypes in comparison with the plastomes of the European sections.

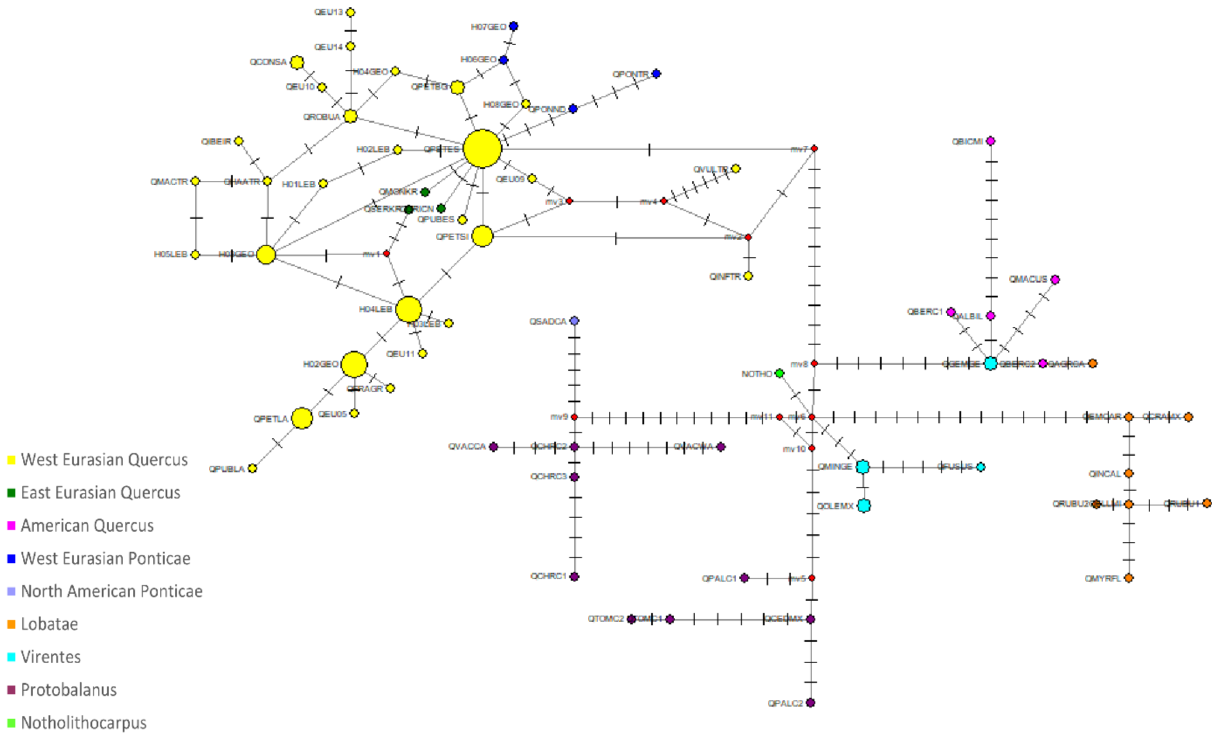


Figure 3.3 Median-joining network based on the two plastid loci (*trn*H-*psb*A and *trn*K-*mat*K) of the 110 sequences of the subgenus *Quercus* belonging to the different sections, and the sequence of the sample of *Notholithocarpus densiflorus* as outgroup. Each sequenced haplotype is represented by a circle, the size of which is proportional to its overall frequency in the dataset (Supplementary File 3). The number of mutational changes between two distinct haplotypes is shown by the lines on the branches. The red diamond represents the median vector. Colorations refers to the taxonomic and geographic affiliations of specimens to *Quercus* sections and macro-regions

In the Neighbor-Net analysis (Fig. 3.4) the low differentiation observed within the Eurasian oaks of section *Quercus*, even between members from western Eurasia and eastern Asia is remarked.

All members of subgenus *Cerris* are placed close to the North American cluster. A huge trunk confirms the main split between North American and Eurasian sections *Quercus* and *Ponticae*. Except for a few divergent sequences, all the Eurasian samples of section *Quercus* are highly mixed and organized in reticulated and little diverging clusters.

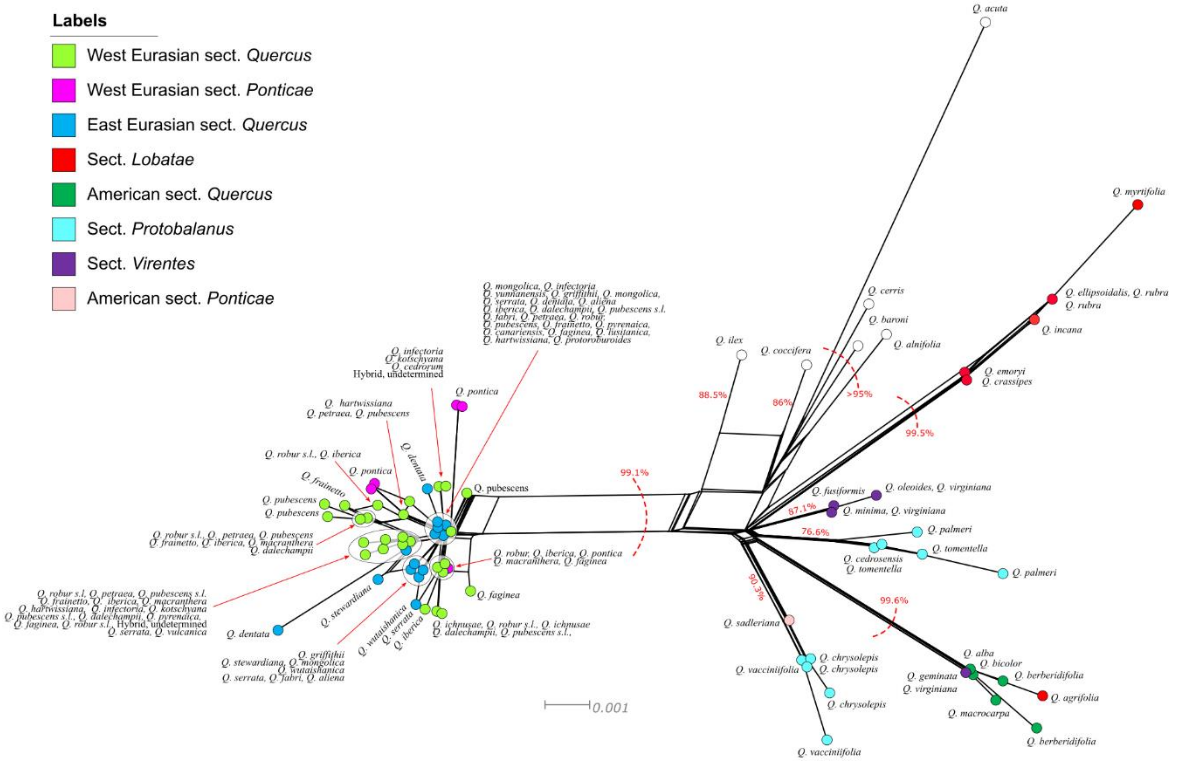


Figure 3.4 Neighbor-Net graph estimated from p-distances with SplitsTree, using the complete alignment from the two plastid loci (*trn*H-*psb*A and *trn*K-*mat*K) sequences of the samples of the different sections of subgenus *Cerris* and *Quercus*. Phylogenetic clade colours correspond to the sections of subgenus *Quercus*; the outgroups are in white colour

In the RAxML tree (Fig. 3.5) rooted between subgenus *Cerris* and subgenus *Quercus* is shown the well-acknowledged sectional differentiation within each subgenus. In subgenus *Cerris*, the two Euro-Med members of section *Ilex* (*Quercus ilex* and *Q. coccifera* from Spain and North Africa) slightly diverge from the other members of the same section (*Q. alnifolia* and *Q. baroni*, belonging to the WAHEA and the East Asian lineages, respectively) and from the two members of section *Cerris* (*Q. cerris*) and section *Cyclobalanopsis* (*Q. acuta*) forming a minor subclade (BS = 57-62). In subgenus *Quercus*, five clades are distinguished with medium-high support (BS = 60-100). Four clades include the North American oaks of subgenus *Quercus* (sections *Quercus*, *Lobatae*, *Protobalanus*, *Virentes*, and *Ponticae*) and the last one collects the Eurasian oaks (sections *Quercus* and *Ponticae*).

Besides the major geographic split between sections *Quercus* and *Ponticae*, sectional differentiation across North American and Eurasian white oaks is fairly solved, despite some cases of intersectional misplacement. These involve a few members of sections *Virentes* and *Lobatae*, and the two only American and Eurasian surviving members of section *Ponticae* (respectively inserted in section *Protobalanus* and Eurasian *Quercus*). The Eurasian white oak clade (BS = 99) is highly unresolved, and no significant differentiation could be observed at the taxonomic or geographic level, even between western and eastern Eurasian samples. Some exceptions are represented by four minor subclades including two samples of *Quercus pontica* (BS = 96), a small group of East Asian oaks (BS = 80), and two local species groups from Lebanon and South-central

Italy (BS = 64-67). It is plausible but weakly supported (BS < 50) to distinguish a clade-basal group of sequences containing both single and shared West and East Eurasian haplotypes.

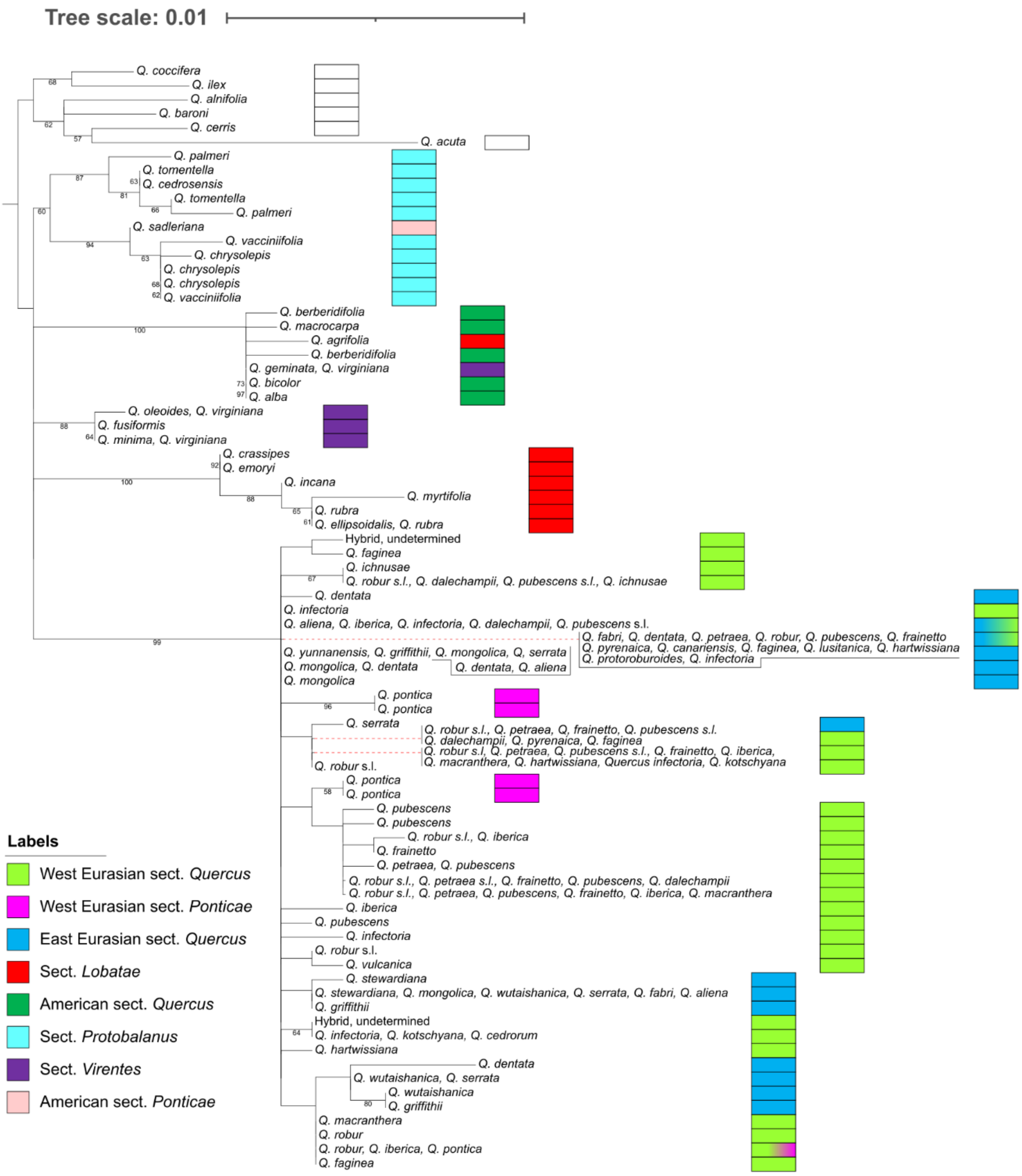


Figure 3.5 RAxML tree of the *trn*H-*psb*A + *trn*K-*mat*K concatenated regions of the investigated samples belonging to subgenera *Cerris* and *Quercus*. Branch support (>50) values are reported above branches. Colorations refers to the taxonomic and geographic affiliations of specimens to *Quercus* sections and macro-regions

3.2 Results of the nuclear microsatellite markers analyses

In total, 1072 samples, belonging to 58 populations of the *Quercus* genus, were analysed by means of 12 microsatellite markers (Supplementary File 4). The markers were successfully amplified and sequenced across all samples, although PCR results for markers PIE239 and PIE258 were not clear. In addition, the binning for these latter two markers showed a significant overlap in bins boundaries, generating indistinct allele size classes, and causing inappropriate allele binning for adjacent alleles. This may be due to mutation in primer binding sites, mutations in regions upstream or downstream of the repetitive motifs, or non-suitable PCR amplification protocols. Therefore, in order to avoid introducing uncorrected values in the study, markers PIE239 and PIE258 were removed from the subsequent analyses. Considering the remaining ten markers analysed, although not all the samples amplified for all the markers, in each locus a proportion higher than 80% of genotyped samples was detected (Fig. 3.6).

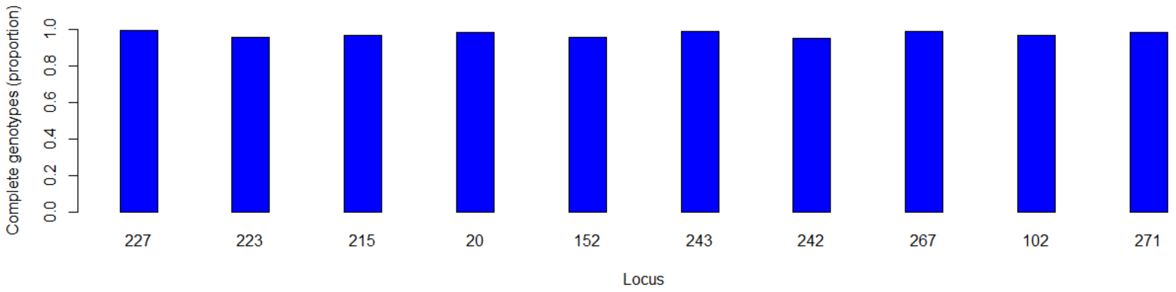


Figure 3.6 Proportion of genotyped samples per locus (PIE)

3.2.1 Results of the Genetic Structure Analysis

According to the structure analysis performed on the entire dataset of the 58 populations analysed by means of the ten microsatellite markers, the number of genetic clusters (K) that best fit the data was 5 (Fig. 3.7).

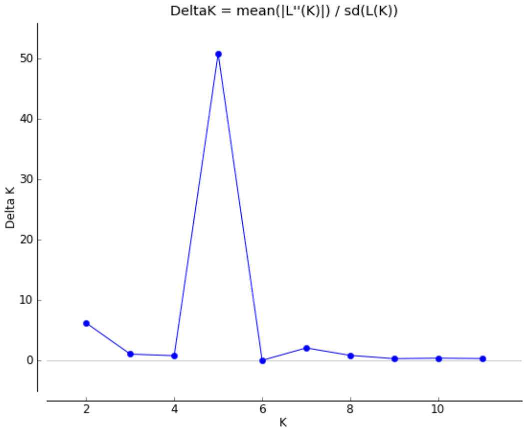


Figure 3.7 Delta K plot representing the most probable number (K = 5) of genetic clusters in the 58 populations of *Quercus* analysed using the Evanno method (2005) implemented in the STRUCTURE HARVESTER program

These five clusters are represented by different colours in the Figure 3.8 and correspond significantly to the main taxonomic groups identified in the populations studied. Moreover, structure analysis allowed to recognize the presence of putative hybrid samples and of individuals not correctly classified from a taxonomic point of view.

The five clusters detected can be defined as follows:

- ✓ **Cluster 1** (depicted in dark pink colour in the bar plot of Figure 3.8)**:** mainly populations of *Quercus pubescens s.l.* with a wide geographic distribution, spread from Iberian Peninsula to Balkan Peninsula, up to northern and central Europe. Hereafter cluster 1 will be referred as PWD (where PWD stands for *Q. pubescens* wide distribution)
- ✓ **Cluster 2** (depicted in sky blue colour in the bar plot of Figure 3.8)**:** mainly populations of *Quercus pubescens s.l.* distributed from central and southern Italy, and in Sardinia Island. Hereafter cluster 2 will be referred as PCSIS (where PCSIS stands for *Q. pubescens* from central and southern Italy, and Sardinia)
- ✓ **Cluster 3 (**depicted in orange colour in the bar plot of Figure 3.8)**:** mainly populations of *Quercus petraea s.l.* including populations of *Q. petraea s.s.*, *Q. banatus*, *Q. dalechampii* (from Serbia and North Italy), and *Q. petraea* subsp. *austrotyrrhenica*
- ✓ **Cluster 4 (**depicted in violet colour in the bar plot of Figure 3.8)**:** populations of *Quercus robur* and *Q. pyrenaica*
- ✓ **Cluster 5 (**depicted in green colour in the bar plot of Figure 3.8)**:** populations of *Quercus frainetto*

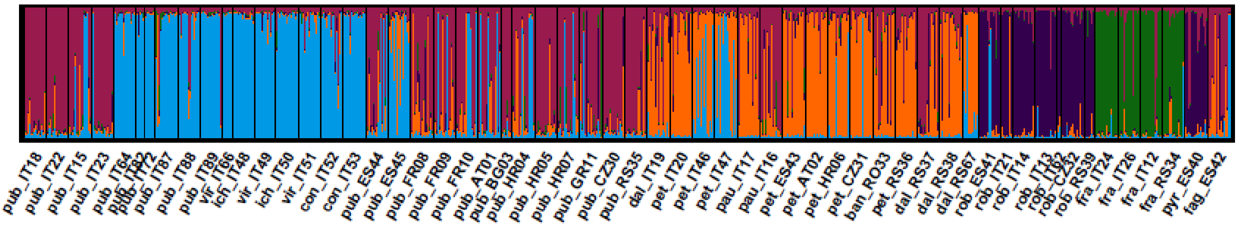


Figure 3.8 CLUMPAK plot (K = 5) describes the genetic structure of the 58 populations of *Quercus* analysed based on the STRUCTURE and STRUCTURE Harvester outputs. Each cluster is represented by a different colour and each vertical bar plot represents an individual and shows individual membership coefficients to the five clusters. A black vertical line separates each population. The name of the populations is identified by a code of three letters that refers to the *taxon* name (ban = *Quercus banatus*, con = *Q. congesta*, dal = *Q. dalechampii*, fag = *Q. faginea*, fra = *Q. frainetto*, ich = *Q. ichnusae*, pau = *Q. petraea* subsp. *austrotyrrhenica*, pet = *Q. petraea*, pub = *Q. pubescens*, pyr = *Q. pyrenaica*, rob = *Q. robur*, vir = *Q. virgiliana*) and four carachters for the identification code of the population

Proportion of membership (q_i) to the clusters of each of the 58 populations is summarized in Table 3.4 and depicted in the stacked bar plot (Fig. 3.9), while the distribution map of the clusters in the populations is shown in Figure 3.10. As hybridization is a well-documented phenomenon in the white oaks, assignation of some populations to a specific cluster was not possible, since genotypes of different clusters were detected in the same population.

Table 3.4 Proportion of membership (q_i) to the five clusters of the 58 populations of *Quercus* analysed. Q_i values ≥ 0.60 are in bold

No. Population	ID Population	No. of samples	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
1	**pub_IT18**	20	**0.843**	0.038	0.097	0.015	0.007
2	**pub_IT22**	20	**0.861**	0.036	0.055	0.025	0.024
3	**pub_IT15**	20	**0.658**	0.245	0.064	0.019	0.014
4	**pub_IT23**	20	**0.862**	0.080	0.022	0.022	0.015
5	**pub_IT64**	20	0.027	**0.937**	0.024	0.006	0.005
6	**pub_IT82**	7	0.018	**0.897**	0.012	0.031	0.042
7	**pub_IT72**	10	0.017	**0.954**	0.012	0.008	0.008
8	**pub_IT87**	20	0.058	**0.821**	0.064	0.009	0.048
9	**pub_IT88**	20	0.110	**0.820**	0.055	0.009	0.007
10	**pub_IT89**	20	0.092	**0.886**	0.009	0.006	0.008
11	**vir_IT66**	8	0.018	**0.966**	0.007	0.004	0.004
12	**ich_IT48**	20	0.022	**0.949**	0.013	0.009	0.007
13	**vir_IT49**	19	0.028	**0.931**	0.022	0.014	0.005
14	**ich_IT50**	20	0.028	**0.941**	0.014	0.008	0.009
15	**vir_IT51**	20	0.058	**0.871**	0.051	0.010	0.010
16	**con_IT52**	20	0.086	**0.848**	0.044	0.018	0.005
17	**con_IT53**	20	0.063	**0.905**	0.017	0.011	0.004
18	**pub_ES44**	20	0.551	0.250	0.138	0.039	0.022
19	**pub_ES45**	20	0.030	**0.651**	0.266	0.038	0.015
20	**pub_FR08**	20	**0.636**	0.030	0.238	0.074	0.023
21	**pub_FR09**	20	**0.611**	0.121	0.185	0.074	0.009
22	**pub_FR10**	20	0.585	0.258	0.113	0.036	0.008
23	**pub_AT01**	20	**0.650**	0.241	0.080	0.021	0.009
24	**pub_BG03**	10	**0.777**	0.033	0.162	0.010	0.018
25	**pub_HR04**	20	0.578	0.202	0.184	0.011	0.024
26	**pub_HR05**	20	**0.802**	0.085	0.059	0.039	0.015
27	**pub_HR07**	20	**0.705**	0.138	0.106	0.011	0.040
28	**pub_GR11**	20	0.588	0.174	0.060	0.092	0.086
29	**pub_CZ30**	20	**0.831**	0.033	0.089	0.037	0.009
30	**pub_RS35**	20	**0.834**	0.023	0.094	0.041	0.009
31	**dal_IT19**	20	0.232	0.041	**0.653**	0.053	0.021
32	**pet_IT20**	20	0.123	0.019	**0.778**	0.069	0.012
33	**pet_IT46**	20	0.021	0.338	**0.629**	0.008	0.004
34	**pet_IT47**	20	0.020	0.456	0.510	0.009	0.006
35	**pau_IT17**	20	0.271	0.038	**0.659**	0.025	0.006
36	**pau_IT16**	20	**0.642**	0.018	0.264	0.044	0.032
37	**pet_ES43**	20	0.112	0.019	**0.802**	0.035	0.032
38	**pet_AT02**	20	0.053	0.020	**0.912**	0.010	0.005
39	**pet_HR06**	20	0.096	0.088	**0.736**	0.067	0.013
40	**pet_CZ31**	20	0.062	0.057	**0.858**	0.017	0.006
41	**ban_RO33**	20	0.270	0.016	**0.606**	0.087	0.021
42	**pet_RS36**	20	0.191	0.015	**0.751**	0.033	0.009
43	**dal_RS37**	20	0.512	0.018	0.321	0.136	0.012
44	**dal_RS38**	20	0.188	0.019	**0.725**	0.057	0.012
45	**dal_RS67**	14	0.022	0.283	**0.683**	0.008	0.004
46	**rob_ES41**	20	0.085	0.119	0.026	**0.755**	0.016
47	**rob_IT21**	10	0.019	0.009	0.107	**0.860**	0.004
48	**rob_IT14**	20	0.039	0.010	0.030	**0.914**	0.005
49	**rob_IT13**	20	0.032	0.023	0.007	**0.932**	0.006
50	**rob_IT62**	4	0.018	0.280	0.049	**0.650**	0.004
51	**rob_CZ32**	20	0.033	0.015	0.037	**0.906**	0.009
52	**rob_RS39**	10	0.025	0.010	0.025	**0.915**	0.025
53	**fra_IT24**	20	0.018	0.036	0.016	0.006	**0.924**
54	**fra_IT26**	20	0.100	0.014	0.034	0.013	**0.840**
55	**fra_IT12**	20	0.132	0.013	0.013	0.006	**0.836**
56	**fra_RS34**	20	0.029	0.041	0.047	0.022	**0.862**
57	**pyr_ES40**	20	0.182	0.025	0.025	**0.758**	0.008
58	**fag_ES42**	20	0.593	0.072	0.210	0.120	0.005

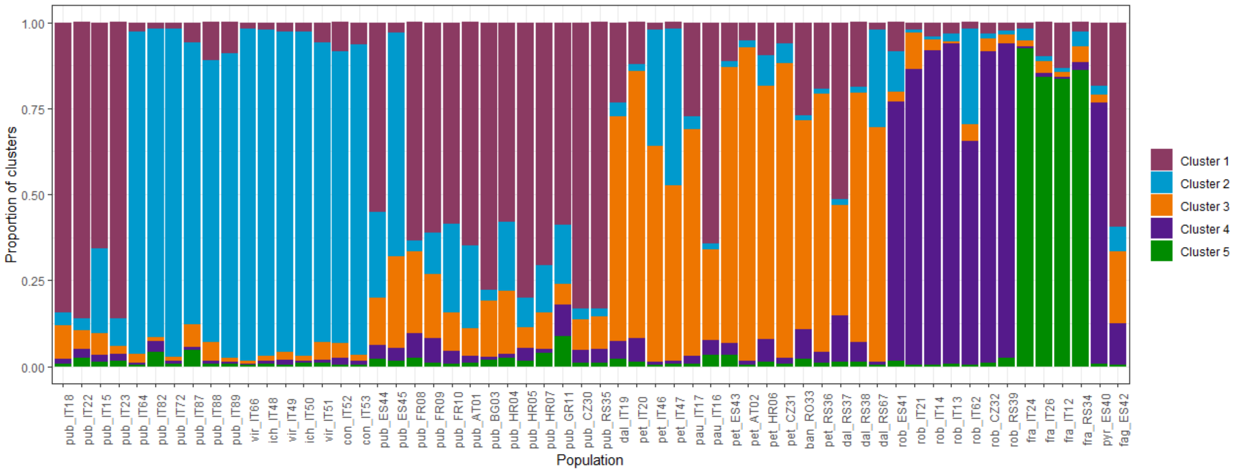


Figure 3.9 Stacked bar plot representing the proportion of membership to the clusters of the 58 populations of *Quercus* analysed

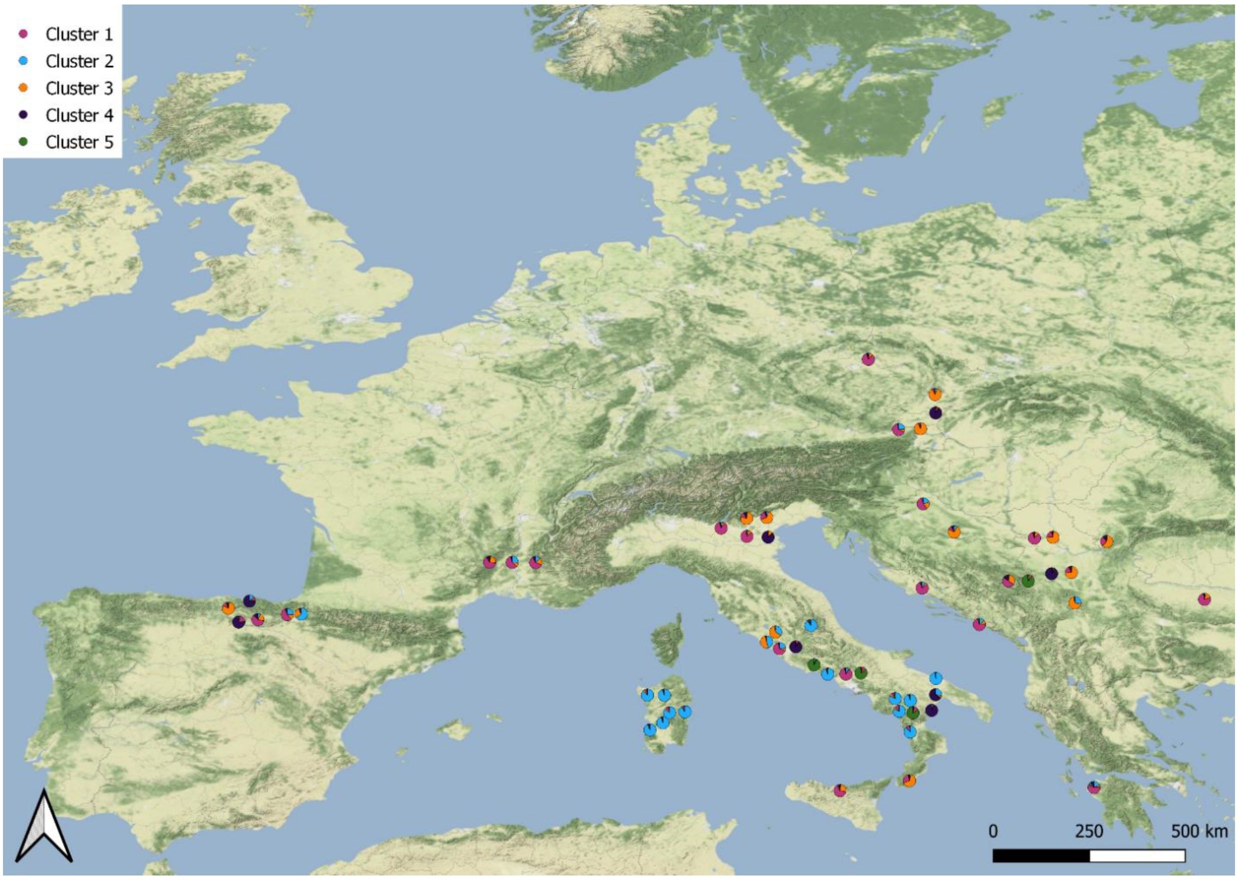


Figure 3.10 Distribution map of the 58 populations of *Quercus* analysed by means of SSRs showing the proportion of each cluster identified according to the structure analysis in each population

A total of 645 out of 1072 samples analysed (60.17%) exhibited membership coefficients (Q values) ≥ 0.90. Among these samples, 87 belonged to cluster 1, 216 to cluster 2, 127 to cluster 3, 154 to cluster 4, 61 to cluster 5. Percentage of the individuals with Q values ≥ 0.90 for each cluster in the 58 populations of *Quercus* sampled is referred in Table 3.5 and the distribution map is shown in Figure 3.11.

Table 3.5 Percentage of the individuals with membership coefficients $Q \geq 0.90$ for each cluster in the 58 populations of *Quercus* analysed. Individuals with $Q < 0.90$ are shown in the mixed clusters group

No. Population	ID Population	No. of samples	% Cluster 1 ($Q \geq 0.90$)	% Cluster 2 ($Q \geq 0.90$)	% Cluster 3 ($Q \geq 0.90$)	% Cluster 4 ($Q \geq 0.90$)	% Cluster 5 ($Q \geq 0.90$)	% Mixed Clusters ($Q < 0.90$)
1	**pub_IT18**	20	50	0	5	0	0	45
2	**pub_IT22**	20	65	0	0	0	0	35
3	**pub_IT15**	20	45	20	0	0	0	35
4	**pub_IT23**	20	70	5	0	0	0	25
5	**pub_IT64**	20	0	85	0	0	0	15
6	**pub_IT82**	7	0	71.43	0	0	0	28.57
7	**pub_IT72**	10	0	100	0	0	0	0
8	**pub_IT87**	20	0	65	0	0	0	35
9	**pub_IT88**	20	0	70	0	0	0	30
10	**pub_IT89**	20	0	90	0	0	0	10
11	**vir_IT66**	8	0	100	0	0	0	0
12	**ich_IT48**	20	0	95	0	0	0	5
13	**vir_IT49**	19	0	84.21	0	0	0	15.79
14	**ich_IT50**	20	0	90	0	0	0	10
15	**vir_IT51**	20	0	65	0	0	0	35
16	**con_IT52**	20	0	60	0	0	0	40
17	**con_IT53**	20	0	90	0	0	0	10
18	**pub_ES44**	20	20	15	0	0	0	65
19	**pub_ES45**	20	0	30	10	0	0	60
20	**pub_FR08**	20	30	0	10	0	0	60
21	**pub_FR09**	20	30	10	10	0	0	50
22	**pub_FR10**	20	20	15	5	0	0	60
23	**pub_AT01**	20	50	15	0	0	0	35
24	**pub_BG03**	10	60	0	0	0	0	40
25	**pub_HR04**	20	30	15	0	0	0	55
26	**pub_HR05**	20	60	5	0	0	0	35
27	**pub_HR07**	20	45	10	0	0	0	45
28	**pub_GR11**	20	30	10	0	5	5	50
29	**pub_CZ30**	20	55	0	0	0	0	45
30	**pub_RS35**	20	80	0	5	0	0	15
31	**dal_IT19**	20	0	0	30	0	0	70
32	**pet_IT20**	20	0	0	45	0	0	55
33	**pet_IT46**	20	0	0	30	0	0	70
34	**pet_IT47**	20	0	15	35	0	0	50
35	**pau_IT17**	20	0	0	45	0	0	55
36	**pau_IT16**	20	20	0	0	0	0	80
37	**pet_ES43**	20	0	0	65	0	0	35
38	**pet_AT02**	20	0	0	80	0	0	20
39	**pet_HR06**	20	0	0	55	0	0	45
40	**pet_CZ31**	20	0	5	70	0	0	25
41	**ban_RO33**	20	0	0	30	0	0	70
42	**pet_RS36**	20	0	0	30	0	0	70
43	**dal_RS37**	20	15	0	15	5	0	65
44	**dal_RS38**	20	0	0	30	0	0	70
45	**dal_RS67**	14	0	0	28.57	0	0	71.43
46	**rob_ES41**	20	0	0	0	55	0	45
47	**rob_IT21**	10	0	0	10	80	0	10
48	**rob_IT14**	20	0	0	0	75	0	25
49	**rob_IT13**	20	0	0	0	90	0	10
50	**rob_IT62**	4	0	0	0	0	0	100
51	**rob_CZ32**	20	0	0	0	75	0	25
52	**rob_RS39**	10	0	0	0	80	0	20
53	**fra_IT24**	20	0	0	0	0	85	15
54	**fra_IT26**	20	0	0	0	0	70	30
55	**fra_IT12**	20	5	0	0	0	80	15
56	**fra_RS34**	20	0	0	0	0	65	35
57	**pyr_ES40**	20	0	0	0	50	0	50
58	**fag_ES42**	20	20	5	5	0	0	70

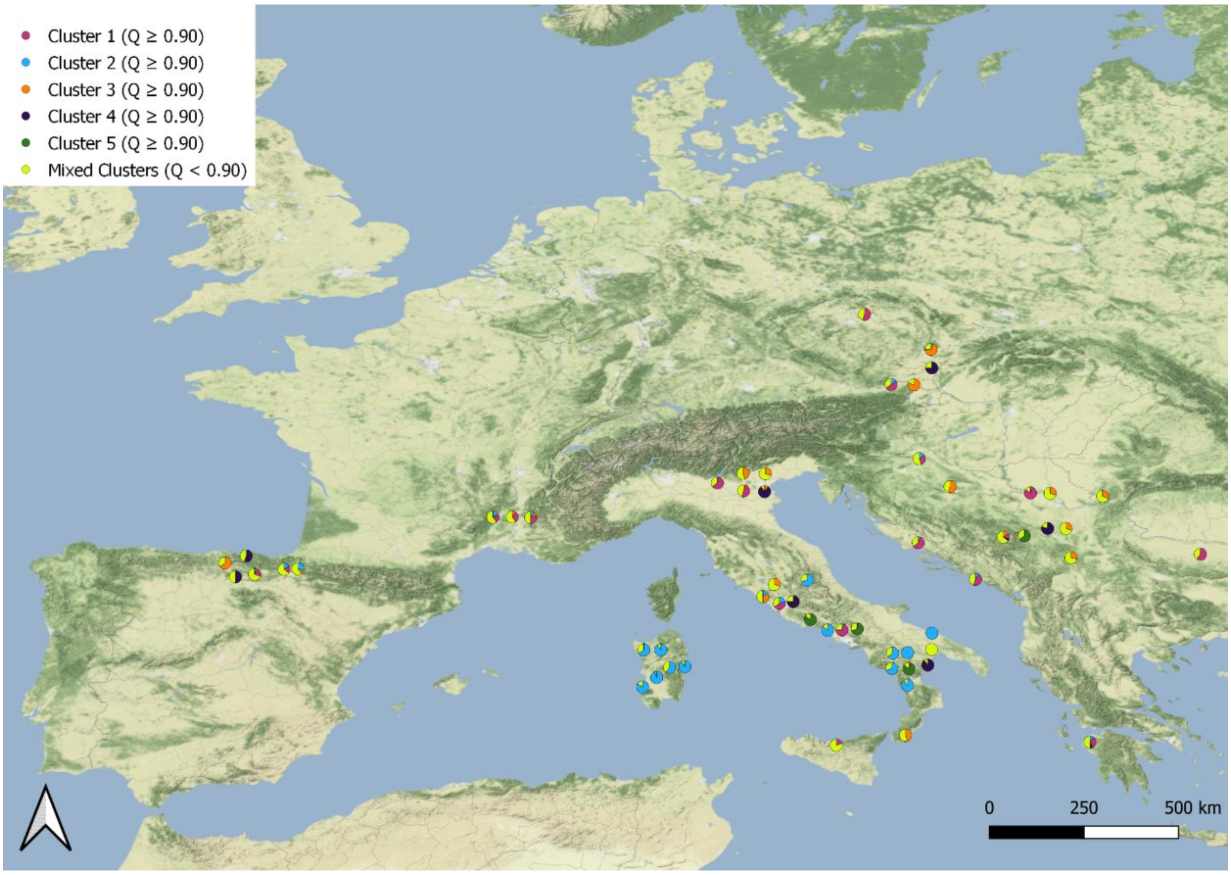


Figure 3.11 Distribution map of the individuals with Q ≥ 0.90 for each cluster in the 58 populations of *Quercus* analysed. Individuals with Q < 0.90 are shown in the mixed clusters group

3.2.2 Results of the Phylogenetic Analysis

The phylogenetic tree based on neighbour-joining method allowed to distinguish the principal taxonomic groups identified in the 58 populations of *Quercus* studied (Fig. 3.12). The main groups recognised reflected considerably the clusters detected by the genetic structure analysis, albeit with some differences. Once again, the populations of *Quercus frainetto* and *Q. robur* formed well separated groups. Even the cluster with the populations of *Quercus petraea s.l.*, including the populations of *Q. banatus*, *Q. dalechampii*, *Q. petraea* subsp. *austrotyrrhenica*, in addition to the populations of *Q. petraea s.s.*, was notably clear. More complex was the grouping of the populations of *Quercus pubescens s.l.*, in fact, different *taxa* from central and southern Italy, and Sardinia Island could be distinguished from the populations occurring in Balkan Peninsula or the populations distributed in other countries from Iberian Peninsula up to northern and central Europe. Not so clear was the collocation of the populations of *Quercus pyrenaica* and *Q. faginea*. Indeed, the first one was associated to *Quercus robur* populations, while the second one was close to populations of *Q. pubescens* from Spain, related from a geographic point of view.

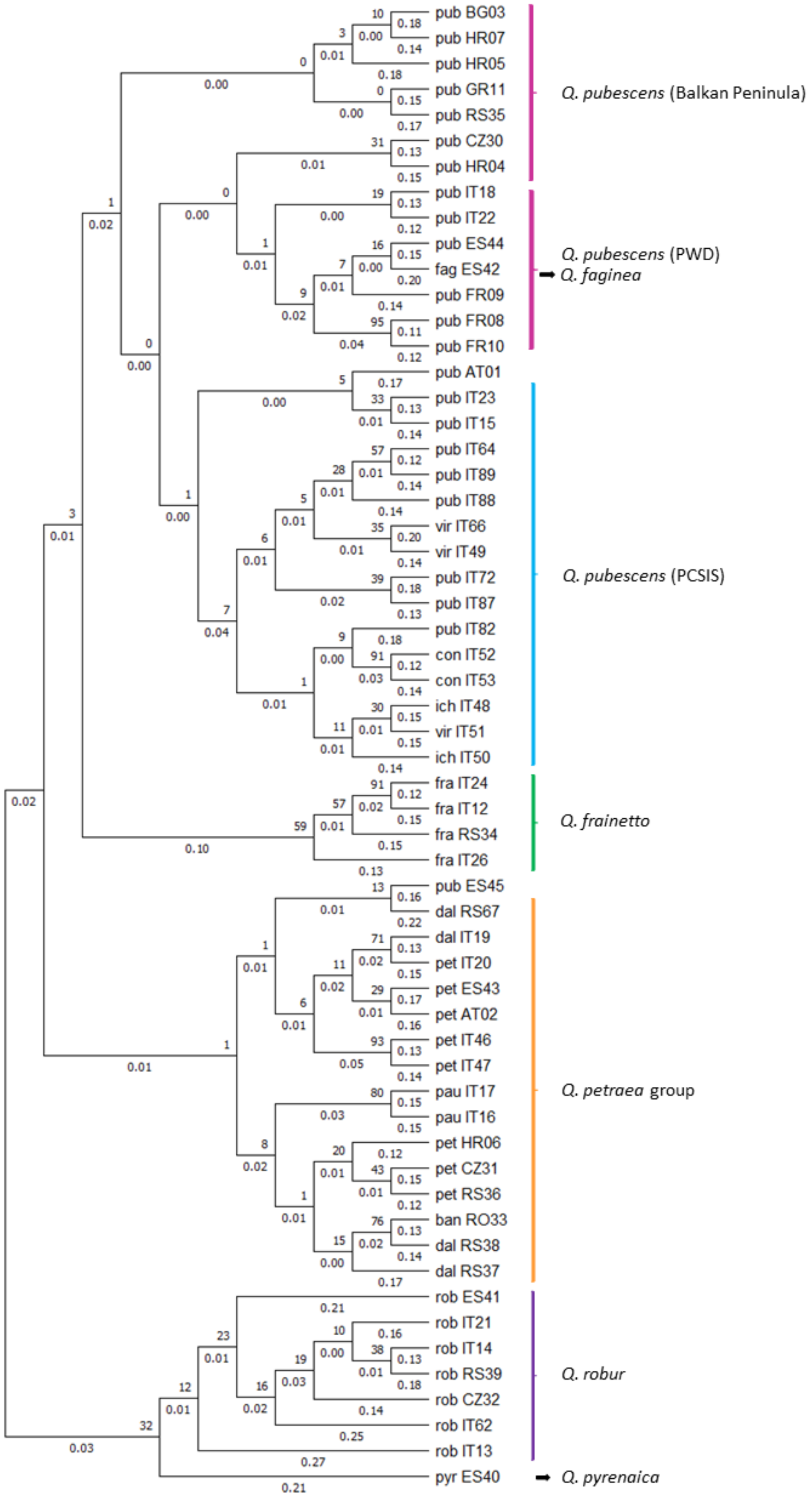


Figure 3.12 Phylogenetic tree of the 58 populations of *Quercus* studied based on the ten markers analysed. The dendrogram was built with MEGA 10.2.6 software using the neighbour-joining method based on the chord genetic distance of Cavalli-Sforza and Edwards (1967). The horizontal branches represent evolutionary changes measured in genetic divergence (number below). The percentage of replicate trees in which the associated *taxa* clustered together in the bootstrap test (999 replicates) is shown next to the branches

3.2.3 Results of the Genetic diversity and differentiation

The genetic statistics were computed for the dataset of 51 populations and separately for the five groups identified from the genetic structure analysis. However, in the latter case, because assignation of some populations to a specific cluster was not possible, since genotypes of different clusters were detected in the same population, only populations with proportion of membership values ≥ 0.60 for a specific cluster were considered.

3.2.3.1 Genetic diversity per locus

The number of alleles per locus (K) over the populations ranged from 13 (PIE227 and PIE267) to 25 (PIE215), for a total of 168 alleles over all the 51 populations analysed. The mean number of alleles (N_a) over the ten loci ranged from 4.157 in PIE227 to 10.000 in PIE152, with an overall mean of 7.200. All the microsatellite markers showed polymorphic information content (PIC) values greater than 0.5, so they can be considered very informative and efficient for evaluating genetic variability and differences between the *Quercus* populations analysed. The lowest PIC value (0.514) was recorded in PIE227 marker, while the highest one (0.900) was observed in PIE152. The informativeness of used loci is summarized in Table 3.6.

Table 3.6 Genetic statistics over the ten loci analysed

Locus	N	K	Observed Alleles	N_a	PIC
PIE227	1009	13	150, 153, 156, 159, 161, 163, 165, 166, 169, 172, 176, 177, 179	4.157	0.514
PIE223	969	14	190, 200, 203, 206, 209, 212, 215, 218, 221, 225, 228, 231, 234, 237	7.627	0.829
PIE215	982	25	187, 188, 190, 191, 193, 194, 196, 197, 199, 200, 202, 203, 205, 206, 208, 209, 211, 212, 214, 215, 217, 218, 220, 221, 224	8.353	0.882
PIE20	996	14	97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 122	4.961	0.650
PIE152	971	20	225, 227, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265	10.000	0.900
PIE243	1002	18	200, 202, 203, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 225, 226, 227, 235	6.196	0.737
PIE242	967	16	100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130	8.961	0.863
PIE267	1003	13	86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110	6.314	0.777
PIE102	982	19	132, 140, 142, 144, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 175, 177	8.196	0.792
PIE271	1000	16	182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 212, 221	7.275	0.821

3.2.3.2 Genetic diversity per population

A total of 17 private alleles (N_p) was recorded over the ten microsatellite markers, in 14 populations out of 51. The populations that exhibited private alleles are represented by: IT48 (*Quercus ichnusae* from Senis, Sardinia Island, Italy), IT53 (*Q. congesta* from Fonni, Sardinia Island, Italy), FR09 (*Q. pubescens* from Montjoyer Drôme, France), AT01 (*Q. pubescens* from Leopoldsberg, Austria), GR11 (*Q. pubescens* from Pinios Lake, Greece), RS35 (*Q. pubescens* from Grgurevci, Serbia), IT19 (*Q. dalechampii* from Euganei Hills, Italy), IT47 (*Q. petraea* from Tolfa, Italy), ES43 (*Q. petraea* from Las Machorras, Spain), RS38 (*Q. dalechampii* from Mt. Suvobor, Serbia), RS67 (*Q. dalechampii* from Kopaonik National Park, Serbia), IT13 (*Q. robur* from Oasis Pantano Policoro, Italy), IT26 (*Q. frainetto* from Aurunci Mountains, Italy), ES42 (*Q. faginea* from Zaldiaran, Spain). The frequency of private alleles in the populations ranged from 0.025 to 0.150 (Table 3.7). The private alleles were confirmed by checking genotyping errors.

Table 3.7 Private alleles and frequencies over the ten loci analysed

No. Population	ID Population	Locus	Allele	Frequency
12	IT48	PIE242	130	0.026
17	IT53	PIE020	122	0.025
21	FR09	PIE227	161	0.050
23	AT01	PIE243	203	0.025
23	AT01	PIE102	132	0.025
28	GR11	PIE102	175	0.026
30	RS35	PIE223	234	0.025
31	IT19	PIE223	190	0.025
34	IT47	PIE215	220	0.050
37	ES43	PIE152	227	0.050
44	RS38	PIE243	200	0.025
45	RS67	PIE271	221	0.036
49	IT13	PIE227	150	0.150
49	IT13	PIE152	225	0.150
54	IT26	PIE102	177	0.025
58	ES42	PIE227	177	0.025
58	ES42	PIE267	88	0.028

The mean number of alleles (N_a) per locus ranged from 5.5 in IT13 (*Quercus robur* from Oasis Pantano Policoro, Italy) to 8.7 in GR11 (*Q. pubescens* from Pinios Lake, Greece), with an overall mean of 7.2. Allelic richness (A_r) showed values ranging from 5.243 in IT13 (*Quercus robur* from Oasis Pantano Policoro, Italy) to 7.882 in GR11 (*Q. pubescens* from Pinios Lake, Greece), with an overall mean of 6.627. The observed heterozygosity (H_o) ranged from 0.523 in ES41 (*Quercus robur* from Leioa, Spain) to 0.800 in RO33 (*Q. banatus* from Oravița, Romania), with an overall mean of 0.693, while the expected heterozygosity (H_e) ranged from 0.629 in IT24 (*Q. frainetto* from Selvapiana Wood, Amaseno, Italy) to 0.805 in ES45 (*Q. pubescens* from Zilbeti, Spain), with an overall mean of 0.733. Values of the frequency of observed heterozygosity (H_o) and expected heterozygosity (H_e) per each population are shown in the bar plot of Figure 3.13.

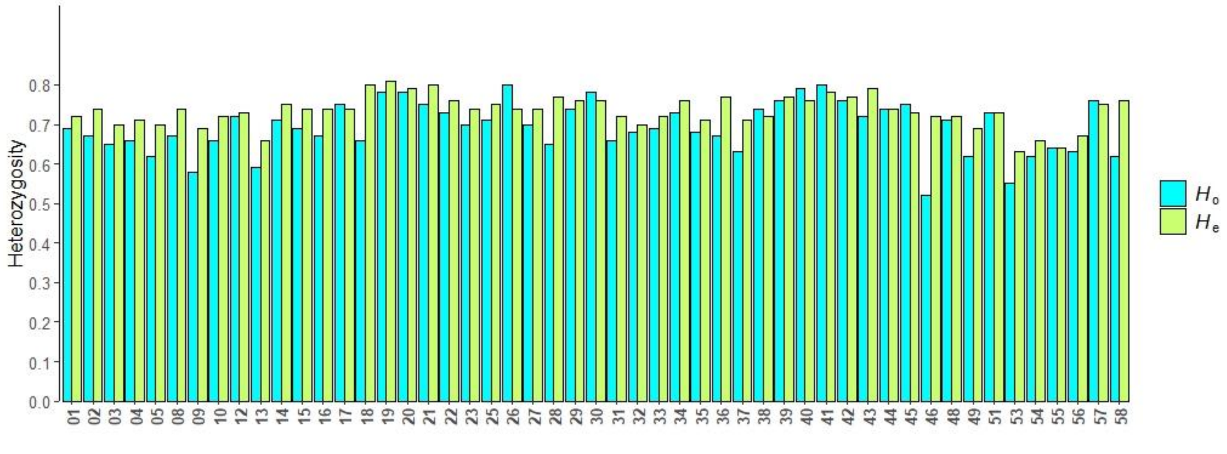


Figure 3.13 Bar plot of the frequency of observed (H_o) and expected heterozygosity (H_e) over the 51 populations of *Quercus* analysed. Bars in cyan indicate frequency of observed heterozygosity (H_o), while bars in light green refer to the frequency of expected heterozygosity (H_e)

Estimation of fixation coefficients (F_{IS}) in the 51 populations of white oaks (Table 3.8) highlighted the presence of a significantly negative F_{IS} value in the population HR05 (*Quercus pubescens* from Mućć, Croatia). Nineteen populations out of fifty-one showed instead significantly positive F_{IS} values, that might be related to inbreeding, presence of null alleles or population substructure, i.e., Wahlund effect (Wahlund 1928).

These populations are represented by: IT22 (*Quercus pubescens* from One, Italy), IT15 (*Q. pubescens* from Insugherata, Italy), IT64 (*Q. pubescens* from Aurunci Mountains, Italy), IT87 (*Q. pubescens* from Sasso di Castalda/Satriano, Italy), IT88 (*Q. pubescens* from Moliterno/Sarconi, Italy), IT89 (*Q. pubescens* from Fosso Cardone/Catarozzolo, Italy), IT49 (*Q. virgiliana* from Pau, Sardinia Island, Italy), IT52 (*Q. congesta* from Fonni, Sardinia Island, Italy), ES44 (*Q. pubescens* from Madotz, Spain), FR09 (*Q. pubescens* from Montjoyer Drôme, France), GR11 (*Q. pubescens* from Pinios Lake, Greece), IT19 (*Q. dalechampii* from Euganei Hills, Italy), IT16 (*Q. petraea* subsp. *austrotyrrhenica* from Pomieri Wood, Sicily Island, Italy), ES43 (*Q. petraea* from Las Machorras, Spain), RS37 (*Q. dalechampii* from Tara Mountain, Serbia), ES41 (*Q. robur* from Leioa, Spain), IT13 (*Q. robur* from Oasis Pantano Policoro, Italy), IT24 (*Q. frainetto* from Selvapiana Wood, Italy), ES42 (*Q. faginea* from Zaldiaran, Spain).

As occurrence of null alleles was detected in several markers in populations with significantly positive F_{IS} (Supplementary File 8), these values most likely rather reflect null alleles than inbreeding in the populations. In addition, these populations showed quite high levels of missing data, that are common in individuals homozygotes for null alleles.

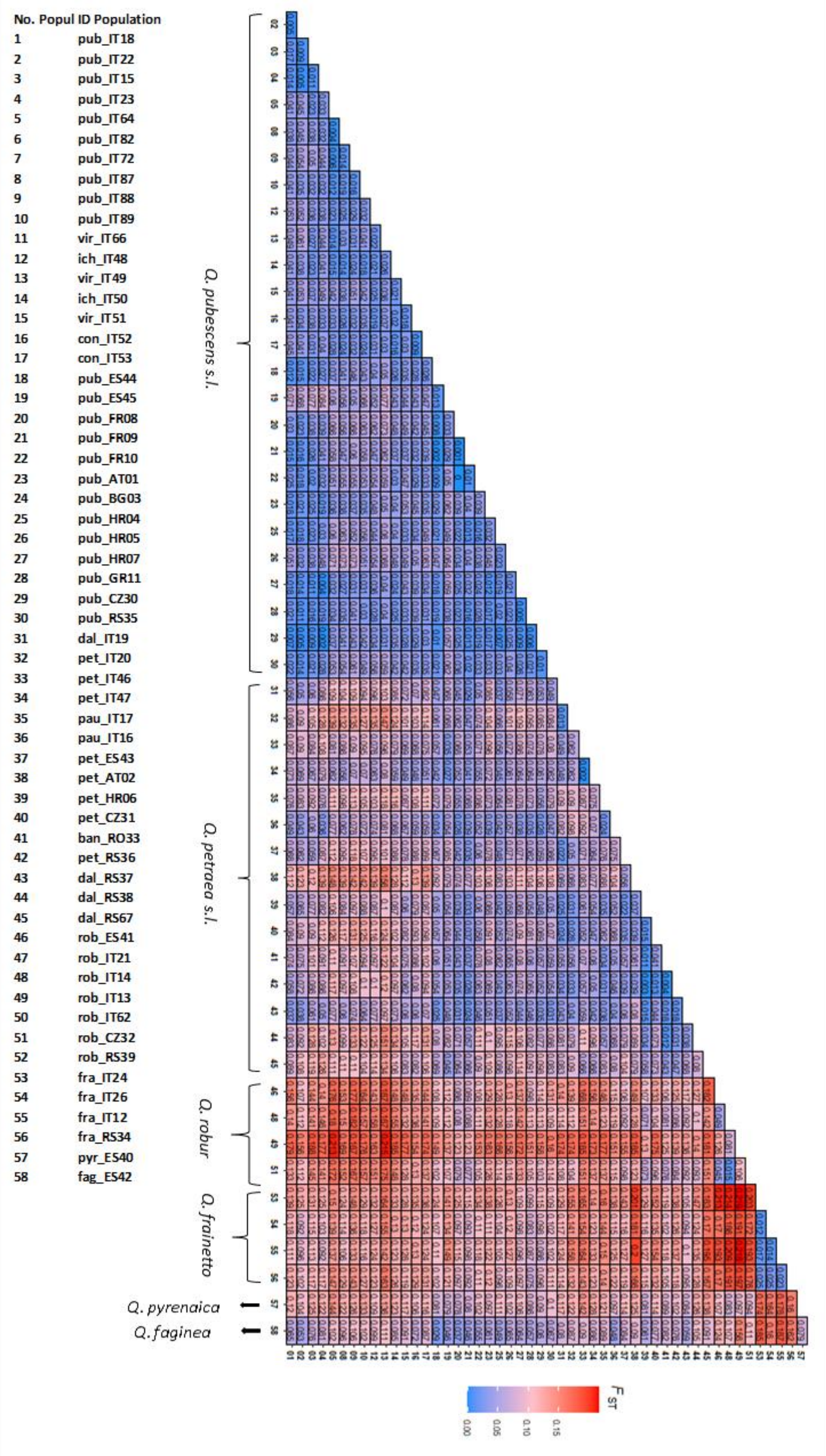
The genetic statistics over the 51 populations analysed are summarized in Table 3.8, while genetic statistics for the five clusters identified from the structure analysis (considering only populations with proportion of membership values ≥ 0.60 for a specific cluster) are provided separately in Supplementary File 9. Seven populations did not fit into the five clusters, as they did not have q_i values ≥ 0.60 for any cluster. These populations are represented by: ES44 (*Quercus pubescens* from Madotz, Spain), FR10 (*Q. pubescens* from Donzère Nord est village, France), HR04 (*Q. pubescens* from Mt. Kalnik, Croatia), GR11 (*Q. pubescens* from Pinios Lake, Greece), IT47 (*Q. petraea* from Tolfa, Italy), RS37 (*Q. dalechampii* from Tara Mountain, Serbia), ES42 (*Q. faginea* from Zaldiaran, Spain).

Table 3.8 Genetic diversity indices in the 51 populations of *Quercus* analysed. N_p: private alleles; N_a: mean number of alleles; A_r: allelic richness (rarefacted for 28 gene copies); H_o: observed heterozygosity; H_e: expected heterozygosity; F_{IS}: fixation coefficient

ID Population	**Taxon**	**No. Samples**	**Missing genotypes (%)**	**N_p**	**N_a**	**A_r28**	**H_o**	**H_e**	**F_{IS}**	**p-value (F_{IS})**	
IT18	*Q. pubescens*	20	0.3 (1.5%)	0	7.3	6.669	0.686	0.715	0.041	0.272	
IT22	*Q. pubescens*	20	0.1 (0.5%)	0	7.7	6.993	0.673	0.739	0.091	0.007	**
IT15	*Q. pubescens*	20	0.6 (3.0%)	0	7.8	7.061	0.651	0.704	0.077	0.041	*
IT23	*Q. pubescens*	20	0.2 (1.0%)	0	8.0	7.078	0.659	0.707	0.069	0.077	
IT64	*Q. pubescens*	20	0.8 (4.0%)	0	7.5	6.784	0.620	0.698	0.114	0.003	**
IT87	*Q. pubescens*	20	1.6 (8.0%)	0	7.5	7.045	0.668	0.734	0.093	0.013	*
IT88	*Q. pubescens*	20	1.4 (7.0%)	0	6.7	6.251	0.576	0.689	0.168	0.000	***
IT89	*Q. pubescens*	20	0.6 (3.0%)	0	6.5	6.069	0.664	0.719	0.078	0.021	*
IT48	*Q. ichnusae*	20	0.1 (0.5%)	1	7.8	7.098	0.724	0.731	0.010	0.669	
IT49	*Q. virgiliana*	19	0.4 (2.1%)	0	6.5	6.099	0.588	0.658	0.108	0.007	**
IT50	*Q. ichnusae*	20	0.1 (0.5%)	0	8.0	7.329	0.709	0.745	0.049	0.141	
IT51	*Q. virgiliana*	20	0.7 (3.5%)	0	6.9	6.395	0.694	0.738	0.062	0.097	
IT52	*Q. congesta*	20	0.9 (4.5%)	0	7.9	7.208	0.668	0.740	0.099	0.005	**
IT53	*Q. congesta*	20	0.5 (2.5%)	1	7.5	6.889	0.752	0.742	-0.013	0.675	
ES44	*Q. pubescens*	20	2.8 (14.0%)	0	8.0	7.606	0.663	0.800	0.176	0.000	***
ES45	*Q. pubescens*	20	0.6 (3.0%)	0	7.6	7.118	0.781	0.805	0.030	0.359	
FR08	*Q. pubescens*	20	0.6 (3.0%)	0	7.2	6.761	0.784	0.795	0.013	0.713	
FR09	*Q. pubescens*	20	1.0 (5.0%)	1	8.3	7.654	0.747	0.801	0.069	0.027	*
FR10	*Q. pubescens*	20	0.2 (1.0%)	0	7.2	6.691	0.727	0.758	0.042	0.224	
AT01	*Q. pubescens*	20	0.4 (2.0%)	2	7.9	7.041	0.696	0.738	0.058	0.154	
HR04	*Q. pubescens*	20	0.0 (0.0%)	0	7.8	7.180	0.710	0.747	0.051	0.132	
HR05	*Q. pubescens*	20	0.1 (0.5%)	0	7.3	6.635	0.799	0.742	-0.080	0.027	*
HR07	*Q. pubescens*	20	1.3 (6.5%)	0	7.5	6.949	0.695	0.735	0.056	0.095	
GR11	*Q. pubescens*	20	0.5 (2.5%)	1	8.7	7.882	0.649	0.766	0.156	0.000	***
CZ30	*Q. pubescens*	20	0.0 (0.0%)	0	8.3	7.605	0.745	0.760	0.020	0.483	
RS35	*Q. pubescens*	20	0.1 (0.5%)	1	7.9	7.229	0.779	0.763	-0.023	0.550	
IT19	*Q. dalechampii*	20	0.1 (0.5%)	1	7.3	6.536	0.658	0.716	0.083	0.031	*
IT20	*Q. petraea*	20	0.0 (0.0%)	0	6.5	5.882	0.675	0.704	0.041	0.294	
IT46	*Q. petraea*	20	0.3 (1.5%)	0	6.5	6.034	0.690	0.721	0.044	0.261	
IT47	*Q. petraea*	20	0.2 (1.0%)	1	7.2	6.788	0.734	0.755	0.029	0.378	
IT17	*Q. petraea* subsp. *austrotyrrhenica*	20	0.5 (2.5%)	0	6.2	5.782	0.681	0.714	0.048	0.200	
IT16	*Q. petraea* subsp. *austrotyrrhenica*	20	0.3 (1.5%)	0	6.4	6.041	0.669	0.765	0.128	0.000	***
ES43	*Q. petraea*	20	0.3 (1.5%)	1	6.3	5.832	0.625	0.704	0.115	0.007	**
AT02	*Q. petraea*	20	0.0 (0.0%)	0	7.6	6.719	0.745	0.719	-0.037	0.326	
HR06	*Q. petraea*	20	0.5 (2.5%)	0	7.7	7.031	0.760	0.767	0.009	0.780	
CZ31	*Q. petraea*	20	0.2 (1.0%)	0	7.4	6.783	0.786	0.756	-0.041	0.291	
RO33	*Q. banatus*	20	0.0 (0.0%)	0	7.5	6.830	0.800	0.781	-0.024	0.518	
RS36	*Q. petraea*	20	0.0 (0.0%)	0	7.8	7.002	0.765	0.767	0.002	0.979	
RS37	*Q. dalechampii*	20	0.0 (0.0%)	0	8.4	7.540	0.725	0.788	0.082	0.009	**
RS38	*Q. dalechampii*	20	0.0 (0.0%)	1	6.7	6.201	0.745	0.739	-0.009	0.885	
RS67	*Q. dalechampii*	14	0.0 (0.0%)	1	5.9	5.900	0.750	0.731	-0.027	0.564	
ES41	*Q. robur*	20	3.1 (15.5%)	0	6.4	6.185	0.523	0.718	0.279	0.000	***
IT14	*Q. robur*	20	0.0 (0.0%)	0	7.1	6.516	0.705	0.723	0.025	0.448	
IT13	*Q. robur*	20	0.2 (1.0%)	2	5.5	5.243	0.623	0.689	0.098	0.025	*
CZ32	*Q. robur*	20	0.1 (0.5%)	0	6.8	6.351	0.734	0.733	-0.002	0.988	
IT24	*Q. frainetto*	20	0.1 (0.5%)	0	6.0	5.411	0.553	0.629	0.124	0.000	***
IT26	*Q. frainetto*	20	0.2 (1.0%)	1	6.0	5.452	0.623	0.659	0.056	0.187	
IT12	*Q. frainetto*	20	0.0 (0.0%)	0	6.3	5.633	0.640	0.638	-0.003	0.951	
RS34	*Q. frainetto*	20	0.4 (2.0%)	0	6.7	5.968	0.629	0.671	0.064	0.141	
ES40	*Q. pyrenaica*	20	0.0 (0.0%)	0	7.0	6.415	0.755	0.751	-0.006	0.886	
ES42	*Q. faginea*	20	2.5 (12.5%)	2	6.9	6.591	0.620	0.756	0.185	0.000	***
mean values		19.86		0.3	7.2	6.627	0.693	0.733	0.056		

3.2.3.3 Genetic differentiation

Pairwise populations differentiation (F_{ST}) showed, as expected, a high genetic differentiation between populations belonging to diverse taxonomic groups (Supplementary File 10). The graphical representation of the genetic differentiation between pairs of populations is shown in the heatmap in Figure 3.14. The highest significant F_{ST} value was recorded between populations IT13 (*Quercus robur* from Oasis Pantano Policoro, Italy) and IT24 (*Q. frainetto* from Selvapiana Wood Amaseno, Italy) ($F_{ST} = 0.2193$, p-value= 0), while the lower significant genetic differentiation ($F_{ST} = 0.0099$, p-value= 0. 048) was documented between populations CZ30 (*Q. pubescens* from Milovice, Czech Republic) and RS35 (*Q. pubescens* from Grgurevci, Serbia).



No. Popul	ID Population
1	pub_IT18
2	pub_IT22
3	pub_IT15
4	pub_IT23
5	pub_IT64
6	pub_IT82
7	pub_IT72
8	pub_IT87
9	pub_IT88
10	pub_IT89
11	vir_IT66
12	ich_IT48
13	vir_IT49
14	ich_IT50
15	vir_IT51
16	con_IT52
17	con_IT53
18	pub_ES44
19	pub_ES45
20	pub_FR08
21	pub_FR09
22	pub_FR10
23	pub_AT01
24	pub_BG03
25	pub_HR04
26	pub_HR05
27	pub_HR07
28	pub_GR11
29	pub_CZ30
30	pub_RS35
31	dal_IT19
32	pet_IT20
33	pet_IT46
34	pet_IT47
35	pau_IT17
36	pau_IT16
37	pet_ES43
38	pet_AT02
39	pet_HR06
40	pet_CZ31
41	ban_RO33
42	pet_RS36
43	dal_RS37
44	dal_RS38
45	dal_RS67
46	rob_ES41
47	rob_IT21
48	rob_IT14
49	rob_IT13
50	rob_IT62
51	rob_CZ32
52	rob_RS39
53	fra_IT24
54	fra_IT26
55	fra_IT12
56	fra_RS34
57	pyr_ES40
58	fag_ES42

Figure 3.14 Heatmap illustrating pairwise F_{ST} between the 51 populations of *Quercus* analysed. F_{ST} values range from 0 (low genetic differentiation) to 1 (high genetic differentiation). The heatmap colour code shows the genetic differentiation from low (blue) to high (red) values

3.2.4 Results of the Analysis of Molecular Variance

The analysis of molecular variance (AMOVA) showed that most of the genetic variation was found within individuals (86.70%). The genetic variation observed among populations was 8.33%, while the lowest variation was recorded among individuals within populations (4.97%) (Table 3.9).

Table 3.9 Analysis of molecular variance (AMOVA) based on the ten loci of 1013 samples belonging to the 51 populations of *Quercus* analysed

Source of variation	Sum of squares	Variance components	Percentage variation
among populations	838.981	0.33297	8.32503
among individuals within populations	3615.417	0.19887	4.97232
within individuals	3422	3.46779	86.70266
Total	7876.398	3.99964	

3.2.5 Results of the Principal Coordinates Analysis

The PCoA scatter plot based on the pairwise population matrix of F_{ST} values (Fig. 3.15) showed a clear separation among *taxa* groups. The grouping pattern of the PCoA analysis corresponded indeed to the taxonomical classification of the species in the populations studied. Except for few populations of the groups of *Quercus pubescens s.l.* and *Q. petraea s.l.*, no overlap was documented among clusters. The geometric distances between all the genotypes analysed revealed a neat discrimination among the four populations of *Quercus frainetto* and the populations belonging to the other *taxa* groups along the second axis of the PCoA scatter plot (12.84% of the variability explained). While the first axis, that explained the 23.96% of the overall genetic variation, highlighted mainly the differentiation among *Quercus robur*, *Q. petraea* group, and *Q. pubescens* group. Not so evident was the collocation of *Quercus pyrenaica* and *Q. faginea* populations, as just one single population per each of the two species was sampled and this has not allowed to distinguish a clear pattern of distribution.

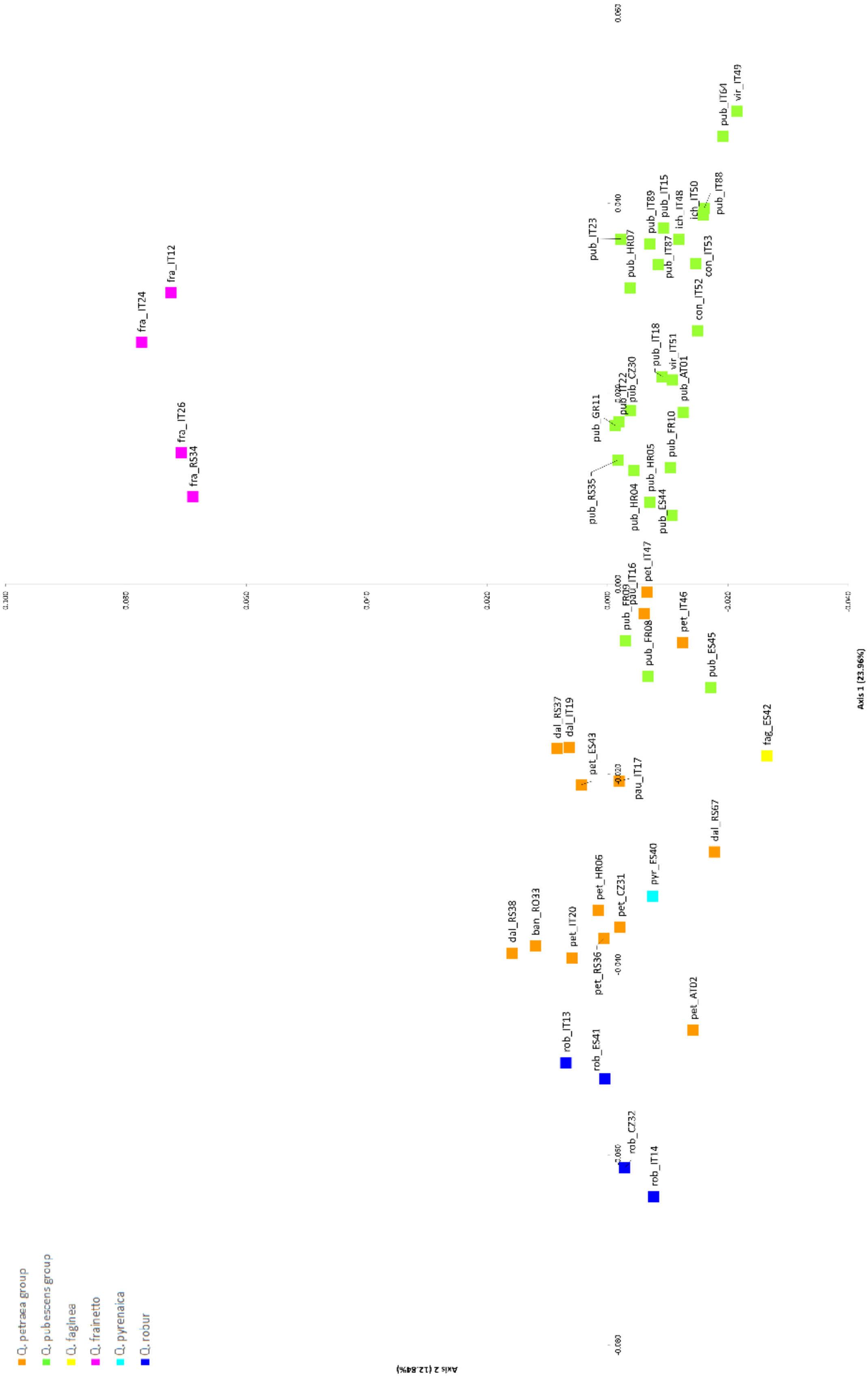


Figure 3.15 Principal coordinate analysis (PCoA) scatter plot of the 51 populations of *Quercus* analysed by means of ten SSR markers. Axes 1 and 2 explain 23.96% and 12.84% of the total variance, respectively. Colours of the squares correspond to the taxonomic groups of belonging of each population

3.2.6 Results of the comparison of geographical and genetic distance

Mantel tests were performed separately on the five clusters highlighted from the genetic structure analysis (considering only populations with q_i values ≥ 0.60 for a specific cluster), and they showed a slightly positive significant correlation between genetic and geographic distances in each of the groups of populations analysed (Fig. 3.16). More in detail, the highest correlation was recorded into the cluster 2 composed primarily by populations of *Quercus pubescens* (PCSIS) (R_{XY} = 0.0322, p = 0.001). Analyses revealed for the other groups the following values of correlation: cluster 1 (R_{XY} = 0.0172, p = 0.001); cluster 3 (R_{XY} = 0.0245, p = 0.001); cluster 4 (R_{XY} = 0.0271, p = 0.001); cluster 5 (R_{XY} = 0.0107, p = 0.010).

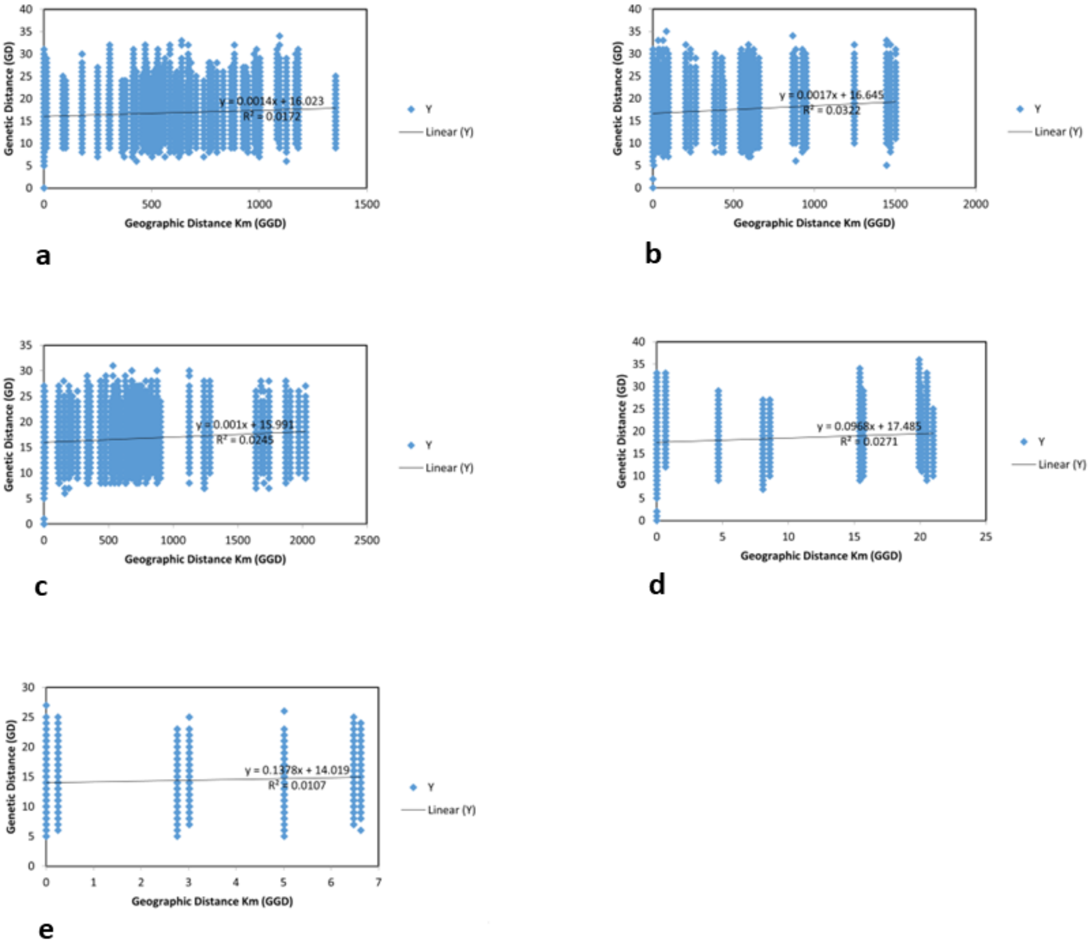


Figure 3.16 Mantel tests showing the correlation between pairwise geographic distance (GGD) and pairwise co-dominant genotypic genetic distance (GD). Each diamond point represents a pairwise comparison. Mantel tests are shown separately for each of the five clusters highlighted from structure analysis. Only populations with q_i values ≥ 0.60 for a specific cluster were considered. The groups are represented by: **a**: Cluster 1; **b**: Cluster 2; **c**: Cluster 3; **d**: Cluster 4*;* **e**: Cluster 5

4 Discussion

Both common ancestry and current patterns of interpopulation genetic exchange have a significant impact on the genetic structuring of plant populations. The diversity of reproductive ecologies, affecting genetic exchange, and the presence of weak reproductive barriers between otherwise morphologically well-defined species lead to the resulting complexity of the genetic structures (Schaal et al. 1998). In the *Quercus* genus, weak reproductive barriers enable gene flow between populations of the same species, as well as between well-diverged species, and as a result, intraspecific population structure is influenced by interspecific genetic exchange following the phenotypic divergence of species (Schaal et al. 1998). Contrary to the nucleome, usually more quickly homogenised at the species/sectional level (Hipp et al. 2020), the maternally inherited plastome allows to understand the population-area relationships and *taxon* histories (e.g., isolation, reticulation, introgression) (Pham et al. 2017).

Based on chloroplast DNA variation, a detailed phylogeographic analysis of the European *taxa* of white oaks (section *Quercus*) was performed. The results achieved through this study have added new and updated knowledge to the only available diversity studies conducted more than 20 years ago, with dated molecular tools (Petit et al. 2002a) and have complemented the current knowledge on plastome phylogeography of the other sections occurring in the Euro-Mediterranean region, sections *Ilex* and *Cerris* (Simeone et al. 2016, 2018; Vitelli et al. 2017). Both two barcoding loci analysed, *trn*H-*psb*A and *trn*K-*mat*K, adequately differentiated the chlorotypes occurring in the studied area. As expected, the variation encountered was moderate to low, and *trn*H-*psb*A showed a higher variability compared to *trn*K-*mat*K (Table 3.1) across all the three sections of the *Quercus* genus investigated in the Euro-Mediterranean region (sections *Quercus*, *Cerris*, and *Ilex*). Among the three sections analysed, the highest values of molecular diversity were observed in the section *Ilex,* as it was revealed from the highest values of uncorrected p-distance, haplotype diversity, and the largest number of haplotypes and Parsimony Informative Characters observed. While, in section *Quercus* comparable estimates with section *Cerris* (characterized by lower number of individuals included) were detected.

The obtained diversity values are consistent with the evolutionary histories of the three sections investigated. In fact, for sections *Cerris* and *Ilex* was assumed an ancient origin in North-East Asia that dates back to the Eocene (40-50 million years ago), in contrast to a more recent colonization of a stock of North American oaks of section *Quercus* into West Eurasia that dates back at 7.5-18 million years ago (Hipp et al. 2020). The range's expansion of sections *Cerris* and *Ilex* in western Eurasia took place in Early Middle Miocene following two different routes: the Tibet-Himalayan Corridor (*Ilex*) and across northern and central Asia (*Cerris*) (Jiang et al. 2019; Simeone et al. 2018). As a consequence of the spread of these two sections in Eurasia, an East-West Eurasian splits resulted, and the differentiation of the main intra-sectional lineages and species groups took place. Therefore, the sections *Cerris* and *Ilex* have had much time to diversify, adapt to different habitats, and differentiate their plastomes, in comparison to the early colonizers of section *Quercus* arrived in Eurasia from northeastern America in more recent times. Focusing on the *taxa* of European white oaks (section *Quercus*) analysed in this study, fourteen total haplotypes in all the studied area were detected. Although the chloroplast genome has a lower evolutionary rate than the nuclear genome, the differentiation observed resulted satisfactory. However, as the haplotypes were each one separated by single mutations (with the only exception of two singlets likely corresponding to geographically isolated samples) a poorly resolved phylogeographic structure resulted (Fig. 3.2). Moreover, due to the maternal inheritance of the chloroplast DNA in angiosperms (Arroyo-García et al. 2002), no species specificity of the haplotypes for the *taxa* was documented. In fact, the most common haplotypes (H01-H04) have been found in almost all the *taxa* investigated, while the rare haplotypes occurred mainly in the isolated populations, as it was observed for the haplotypes H10, H11, and H12, found in Sardinia and Sicily islands, and Apulia region (southern Italy), respectively. Most populations were fixed for one haplotype to prove a significant homogeneity across the populations from the same area. The most frequent haplotypes (H01 and H02) had a more central eastern distribution in the studied area, in contrast to the second two most common haplotypes (H03 and H04) that were mainly spread in the central western part of the sampled area. Haplotype H04, however, showed a discontinuous distribution, and it was found across the Italian and Iberian Peninsulas, in Morocco and in Bulgaria. Despite its presence in Balkan Peninsula restricted to one single Bulgarian sample, it can be assumed a wider distribution of this haplotype, which has not been detected because of a sparsely extended sampling in the Balkan Peninsula and over. Haplotype H05 was exclusively found in the population of *Quercus pubescens* from Muć (Croatia) and it was not recorded in other populations from the same area. This could be due to the isolation of the population, although no sufficient data are available to infer conclusions. Interestingly, haplotypes H06, H07, H08, H09, H10, H11, and H12 were exclusive to the central southern Italy, major islands (Sicily and Sardinia) included, and while having different geographic distributions, they all prove the role of glacial refugia of the southern Italy during Pleistocene (Brewer et al. 2002; Petit et al. 2002a). In fact, although the number of Italian samples was higher in comparison with the other countries, the presence of high levels of chloroplast diversity (eleven haplotypes), mainly found in the southern part of the country, can be related to the Quaternary southward range contractions. The two remaining rare haplotypes (H13 and H14) were found in the population of *Quercus faginea* from Cordoba (Spain), that showed three different haplotypes in the three samples analysed, proving the heterogeneity of the population of belonging. As it is shown in Figure 3.2, the central haplotypes (H01, H03, H04, H06) in the network were among the most prevalent and spread across the populations sampled, supporting the hypothesis that these belonged to the ancestral populations (Golding 1987; Castelloe and Templeton 1994). Despite the use of a different technique, the most common chlorotypes identified in this study could be assigned to the lineages recognised in previous large-scale studies, based on the PCR RFLP method (Petit et al. 2002a). The maternal lineages recognised by Petit et al. (2002a) A, B, C, and E could be represented by several chlorotypes found in the current study. In fact, the lineage A includes haplotypes found in North and central Europe (North Spain and Italian Peninsula included) to the Balkans; the lineage B includes haplotypes spread in Morocco, Iberian Peninsula and up to France, Great Britain, central and North Europe; the lineage C includes haplotypes found in North Spain, Italian Peninsula and major islands, to central, North and eastern Europe; while the lineage E is restricted to southeastern Europe and the Italian Peninsula (major islands included). According to other studies (Grivet et al. 2006; Neophytou and Michiels 2013), this work confirms the negligible phylogeographic structure in the European white oaks due to the recent recolonization of central Europe by a limited number of chlorotypes, and a more evident structuring for the regions with Mediterranean climate and for the refugial sites. In fact, as the oaks in the Mediterranean area survived in several refugia, an *in situ* differentiation of cpDNA during the ice age and likely for the remainder of the Quaternary took place. Consequently, a patchy distribution and significant phylogeographic structure with well differentiated lineages occurring relatively close to each other and a high total chlorotypic variation resulted (Grivet et al. 2006; López de Heredia et al. 2007; Neophytou et al. 2011). In this scenario, the Italian Peninsula has been a crossroads of post-ice-age recolonization pathways, as proven by the high number of haplotypes found, as well as a hotspot of chloroplast DNA diversity for the European white oaks.

In a global context of the *Quercus* genus a clearer phylogeographic structure appeared analysing the different sections of the genus, as emerged from the network (Fig. 3.3), the neighbour-Net graph (3.4), and the RAxML tree (Fig. 3.5), that show a high level of intersectional differentiation. The topology of the RAxML tree rooted between the two *Quercus* subgenera matches the most recent reconstructions obtained with more numerous or powerful plastid markers (five DNA regions: Yang et al. 2020; over 200 coding and non-coding loci from RNA-seq data: Yang et al. 2021; whole genome sequencing: Zhou et al. 2022) and complements the recently perceived evidence derived from nuclear data (RAD-sequencing; Hipp et al. 2020) of a complex evolution of the entire genus. The RAxML tree highlights the relationships found between the Eurasian white oaks and the *Ilex* Euro-Med lineage. In fact, this latter lineage may be among the oldest diverging *Quercus* plastomes, as first proposed in Simeone et al. (2016), and the result of an ancient cross-sectional oak lineage (Yang et al. 2021). The phylogenetic reconstructions of this study also distinguished between New World and Old World members of the same evolutionary lineage, i.e., sections *Quercus* and *Ponticae*, demonstrating that there must have been a geographic differentiation in their primordial members predating divergence and manifestation of modern *taxa*. Furthermore, the non-monophyly observed in some sections could be due to the chloroplast capture via hybridization in the early diversification of the genus (Manos and Hipp 2021). In section *Ponticae*, for instance, it has been supposed that the relict species *Quercus sadleriana* survived by introgressing plastomes from sympatric *Protobalanus* members (hence, its sister position to one of the two sublclades in the RAxML tree); likewise, its Caucasian sister (*Q. pontica*) introgressed plastomes from different sources of sympatric (West Eurasian) members of section *Quercus* in more recent times (Hipp et al. 2020). The current work allowed to confirm that in the Eurasian white oaks signatures of a distant past coexisting with more recent, still unfixed genetic diversity, bring to a little differentiated plastome. This could be due to the origin of the group from a limited genetic source and the only recent distribution across the continent and in highly heterogeneous, moulding landscapes.

Such reduced plastome variability is matched by a greater nucleome variability, that however does not reflect the considerable morphological variability responsible for the many ecomorphotypes and the taxonomic confusion in the European white oak group. In this study the genetic knowledge of the European *taxa* of white oaks has been improved, providing a better understanding of phenotypic and taxonomic diversity, and addressing the major critical issues concerning mainly the *taxa* of the *Quercus pubescens* group. In fact, mainly for this latter group, it is still open the debate about the possibility of keeping all the pubescent oak *taxa* at the species or subspecies ranks or whether to consider the phenotypic diversity observed as included in the morphological variability pattern of a single widely distributed species (e.g., a pan-European *Quercus pubescens* Willd.) (Di Pietro et al. 2021). The nuclear microsatellite markers analyses turn out to be an efficient tool to deepen the genetic variability among similar *taxa*. Based on the genetic structure analysis performed in this study, the most probable number of clusters in the populations analysed was 5 (K=5) (Fig. 3.7). Although some samples showed genotypes of different clusters (putative hybrids), as well as some populations were not genetically homogeneous, the 60.17% of the samples analysed exhibited membership coefficients (Q values) ≥ 0.90, so they could be considered as genetically "pure". It is therefore plausible to recognize the existence of five genetically distinct groups, to which the twelve *taxa*, identified based on morphological features, may be lead. According to this analysis, a clear lack of correspondence between the relevant morphological variability and the observed genetic variability has emerged. In fact, not all *taxa* (especially those belonging to the *Quercus pubescens* group (e.g., *Q. congesta*, *Q. ichnusae*, *Q. virgiliana*), as well as to the *Q. petraea* group (e.g., *Q. banatus*, *Q. dalechampii*, *Q. petraea* subsp. *austrotyrrhenica*)) recognized by local floras or phytosociological syntheses (mainly in southern Italy) resulted genetically separated. Not surprisingly, a clear classification emerged for the populations of *Quercus frainetto* (cluster 5) and *Q. robur* (cluster 4), for which genetically well-distinguishable groups were identified and, in both cases, high values of proportion of membership (q_i) to the respective clusters resulted (Table 3.4). This confirms that *Quercus frainetto* and *Q. robur* are two genetically well characterised species. Unexpectedly, the same genotype observed in the *Quercus robur* populations was also found in the only population of *Q. pyrenaica* (ES40), which although morphologically quite distinct from the first group, showed $q_i = 0.758$ for the cluster 4 (Table 3.4). A deeper study will be needed to better understand this pattern, as up to this examination no clear explanation has been found. The analysis of microsatellite markers did not reveal a clear genetic pattern for the *Quercus faginea* population (ES42), as shown by the diversity of genotypes found in the individuals sampled ($q_i = 0.593$ for cluster 1, $q_i = 0.210$ for cluster 3, $q_i = 0.120$ for cluster 4) (Table 3.4), thus it can be assumed a mixture genotype or a low resolution of the microsatellites markers for this species. While, interestingly, this analysis has solved, simplifying it, the taxonomic classification of *taxa* belonging to the collective group of *Quercus petraea*. According to this study, the populations of *Quercus dalechampii* from the Balkan Peninsula (RS37, RS38, RS67) and northern Italy (IT19) may be lead, with support of high proportion of membership values (Table 3.4), to the *Q. petraea* group (cluster 3). As a consequence, this result allows to confirm the identification of the *Quercus dalechampii taxa* from the Balkan Peninsula and northern Italy with the *Q. petraea* species. Thus, according to the taxonomic revision proposed by Di Pietro et al. (2012), the name *Quercus dalechampii* Ten. is to be applied to a species belonging to the *Q. pubescens* complex and cannot be used to refer to populations of *Q. petraea*. Similarly, the only population of *Quercus banatus* (RO33) in the sampling showed high proportion of membership value (0.606) for the cluster of the species of *Q. petraea,* so according to this analysis the species rank for this *taxon* is not supported and *Q. banatus* should be led to *Q. petraea* complex. For the two populations from the central Italy, classified based on the morphological traits as *Quercus petraea* (IT46 and IT47), lower values of proportion of membership to the cluster 3 were recorded, to prove the heterogeneity of the populations, as well as a probable co-existence of different *taxa* in the same woods (a high proportion of membership to the cluster 2 was indeed recorded, mainly for the population IT47). In addition, the genetic structure analysis did not support the recognition of the *Quercus petraea* subsp. *austrothyrrenica* taxon. In fact, although for the population IT16 from Sicily Island, the possibility of a sampling in a mixed wood can be supposed ($q_i = 0.642$ for cluster 1, $q_i = 0.264$ for cluster 3), the population IT17 from Calabria region (southern Italy) exhibited a clear appartenence to the cluster of *Quercus petraea* ($q_i = 0.659$), showing no significant differences with the type. While, for the population of *Quercus dalechampii* from Tara Mountains (RS37) could be assumed a sampling of two different *taxa* in the same wood, one belonging to *Q. pubescens* complex (cluster 1, q_i = 0.512) and the other to *Q. petraea* complex (cluster 3, q_i = 0.321). However, the most significant aspect that emerges from the genetic structure analysis concerns the *taxa* belonging to the collective group of *Quercus pubescens*. According to this analysis, the multiplicity of *taxa* identified and recognized on the basis of morphological traits (i.e., *Quercus congesta*, *Q. ichnusae*, and *Q. virgiliana*) is not supported by the observed genetic variability, which instead is expressed in only two clusters. The two groups differentiate the populations of the collective group of *Quercus pubescens* mostly according to a geographic distribution criterion. In particular, albeit with exceptions, there appears to be a cluster (cluster 2) inclusive of *taxa* (*Quercus congesta*, *Q. ichnusae*, *Q. pubescens*, and *Q. virgiliana*) from central southern Italy, including Sardinia Island, to which belongs, even if with lower proportion of membership value (q_i = 0.651), also the Spanish population of *Q. pubescens* (ES45) and a cluster (cluster 1) inclusive of the other populations of *Q. pubescens*, that shows a wider geographic distribution in the studied area, from Iberian Peninsula to Balkan Peninsula, up to northern and central Europe. According to our results all these pubescent oak *taxa* recognized at the rank of species in local floras (Brullo et al. 2001; Bacchetta et al. 2009; Biondi et al. 2010) are not supported from the genetic analysis. For instance, focusing on Sardinia Island, no genetic differences among the endemic *taxon Quercus ichnusae* (populations IT48 and IT50), with the co-occurrent *taxa Q. virgiliana* (populations IT49 and IT51) and *Q. congesta* (populations IT52 and IT53), reported in the taxonomic and phytosociological literature for Sardinia, can be distinguished, and as all these records belong to the same cluster (cluster 2) (Fig. 3.8; Table 3.4), they should be summarized to one single *taxon*. Furthermore, since pure individuals belonging to cluster 2 were also found in other populations, mainly from central southern Italy (except for population ES45 from Spain), the presence of an endemic Sardinian *taxon* could not be confirmed. Interestingly, the proportion of membership values to the cluster of belonging (cluster 2) of the six populations from Sardinia (IT48-IT53) were considerably high to prove the high dregree of “genetic purity” of these populations, principally due to the geographic isolation. At the same cluster belong also the populations of *Quercus pubescens* from central Italy, IT64 and IT82, as it is shown from the high values of q_i, 0.937 and 0.897, respectively. The remaining populations of *Quercus pubescens* with a wider geographic distribution belonged instead to the cluster 1 and differently from the other groups of *taxa*, proportion of membership values were notably lower, to reveal a significant gene flow among populations (Table 3.4). Based on the membership coefficients (Q values), that identify the belonging of each sample to a cluster, emerged that the higher percentage (≥ 90%) of the individuals with membership coefficients $Q \geq 0.90$ for each cluster (corresponding to the more genetically pure samples), has been observed in the populations of *Quercus pubescens* (IT72, IT66) from Basilicata and Apulia regions in southern Italy, in the populations of *Q. ichnusae* (IT48, IT50) from Sardinia Island, in the population of *Q. congesta* (IT53) from Sardinia Island, and in the population of *Q. robur* (IT13) from Basilicata region in southern Italy (Fig. 3.11; Table 3.5). These results not only confirm the important role of the geographic isolation in reducing gene flow and in preserving the original genetic diversity of the populations, but also show that the differences found between groups of pubescent oaks probably express the gene flow to which the populations are subjected. The main clusters recognised by the genetic structure analysis reflect considerably the groups identified in the phylogenetic analysis. However, the reduced amount of genetic changes, represented by the short length of branches of the tree, has not provided good support for many terminal clades as well as several internal nodes, highlighting once again the low genetic variability in the European white oaks, as well as the resultant complexity in the classification. Nevertheless, distinct clusters have been identified and specific phylogeographic and taxonomic patterns have been recognised. The groups of the populations of *Quercus frainetto* and of *Q. robur* appeared well separated, and a weak phylogeographic signal was observed, suggesting ancient divergence for the *taxa* of both groups. As emerged also in the genetic structure analysis, the population of *Quercus pyrenaica* clustered with the *Q. robur* group, although at a different level of clustering in comparison with the other populations. In addition, the group of *Quercus petraea taxa* did not cluster populations according to the different putative *taxa*, but mainly following a geographic pattern. In fact, a subcluster of this group, including the populations from Balkan Peninsula in addition to the two populations of *Quercus petraea* subsp. *austrotyrrhenica* from Calabria region (southern Italy) and Sicily Island, was distinguished by a subcluster including populations with a central western distribution in the studied area. As expected, the clustering for the *Quercus pubescens* group resulted more complex. In fact, although subclusters have emerged from the analysis, the short length of the branches has provided a weak support to the recognition of well separated groups. The first group identified comprised the populations of *Quercus pubescens* from Balkan Peninsula. The second level of clustering brought together different *taxa* of the *Quercus pubescent s.l.*, which could be separated in two further subclusters, one including the populations with a wider geographic distribution, and the other including the populations from central southern Italy and Sardinia Island. Interestingly into this latter group, populations of *Quercus congesta*, *Q. ichnusae*, *Q. virgiliana*, and *Q. pubescens* grouped together, and although sometimes the same *taxa* created subclusters (e.g., *Q. virgiliana* IT66-IT49; *Q. congesta* IT52-IT53), the elevation at the specific rank cannot be supported. Even according to the phylogenetic classification, the population of *Quercus faginea* (ES42) clustered with the populations of *Q. pubescens,* showing affinity mainly for the population di *Q. pubescens* (ES44) from the same geographic area. Based on the results of both genetic structure analysis, as well as phylogenetic analysis, it can be confirmed the complex genetic diversity in the *taxa* of the European white oaks. In fact, despite the reduced, well-fixed, and identifying genetic diversity of some groups (e.g., *Quercus frainetto*, *Quercus robur*), the variability into the collective groups of *Q. pubescens*, but also of *Q. petraea*, results much higher. Focusing on the single populations, the analysis of the parameters of genetic diversity estimated in this study has highlighted the lower value of allelic richness (5.243) in the population of *Quercus robur* from Basilicata region, in southern Italy (IT13). This result is not surprising as this population represents one of the southernmost populations of *Quercus robur* in Italy, moreover due to its geographic isolation can easily be expected a reduced gene flow. Differently, the highest value (7.882) of allelic richness was recorded in the population of *Quercus pubescens* from Pinios Lake in Greece, where a greater possibility of gene flow among populations can be assumed.

The analysis showed only a significantly negative F_{IS} (inbreeding coefficient) value (Table 3.8) across the populations studied that could indicate hybridization in the population of *Quercus pubescens* from Muć in Croatia (HR05). Differently, positive F_{IS} values were recorded in nineteen populations belonging to different *taxa* and from different provenances. However, the presence of inbreeding in these populations cannot be confirmed as these values might be related to presence of null alleles or population substructure (Wahlund effect). In addition, the pairwise populations differentiation (F_{ST}) values confirm a significant diversity among the populations of *taxa* genetically well distinguished (e.g., *Quercus frainetto*, *Q. petraea*, *Q. pubescens*, *Q. robur*), and a weaker differentiation among similar *taxa*, although among the *taxa* of the collective groups of *Quercus pubescens* and *Q. petraea* a higher internal differentiation was observed in comparison to that of the other groups (Fig. 3.14). This genetic diversity cannot be ignored, nevertheless the values recorded into these two groups do not support the elevation at the specific rank of the *taxa* included. The Principal Coordinate Analysis has corroborated what emerged from the other analyses, in fact four main groups represented by *Quercus frainetto*, *Q. robur*, *Q. pubescens*, and *Q. petraea* resulted, although into the two latter groups, *taxa* could be separated in subclusters.

The correlation between genetic and geographical distance among populations of the different groups, identified according to the genetic structure analysis, revealed by the Mantel test was found to be positive and statistically significant, however very low (Figure 3.16). In fact, most of the genetic diversity found was observed within single individuals (86.70%) followed by genetic diversity among different populations (8.32%) and among individuals within the same populations (4.97%) (Table 3.9).

5 Conclusion

In this study an extensive analysis on the chloroplast and nuclear genetic diversity and differentiation across European *taxa* of white oaks (*Quercus* L. section *Quercus*) was performed. Besides the well-documented unresolved taxonomic classification of this group, a pronounced discrepancy between plastid and nuclear data was recorded. Both the young history of the European white oaks and the repeated events of hybridisation and introgression are responsible for a group that is not still genetically fixed and resolved.

The phylogeographic analyses based on the study of the chloroplast DNA variation allowed to confirm the relatively recent colonization of the section *Quercus* in Eurasia, that dates to 7.5-18 million years ago, and the subsequent quick diversification rates, due to the large niche availability of a moulding climate and territories (Hipp et al. 2020). The little differentiated plastome of this oaks group, documented by the low degree of diversity found in the fourteen haplotypes identified in the studied area, is in agreement with the founder effect. In fact, Pleistocene climate changes probably affected the white oaks more than sections *Ilex* and *Cerris* distributed in the same geographic area, further depleting the plastid molecular diversity. Natural barriers as major mountain systems and sea acted to preserve and/or promote further diversification. Furthermore, the local persistence of ancient variants and overlap of derived variants, as well as the genetic drifts, led to the lack of a distinct phylogeographic structure in the plastome of the Eurasian section *Quercus*.

Compared to this reduced plastome variability is a greater nucleome variability, that however, according to the results of this study, does not reflect the notable morphological variability responsible for the many ecomorphotypes and the taxonomic issues in the European white oaks group. In this study, a clear identification of the groups of *Quercus frainetto* and *Q. robur* resulted, while no genetic evidence on the recognition of some *taxa* included in the collective groups of *Quercus pubescens* as well as of *Q. petraea* has been found. More in detail, the already notoriously difficult classification of the *Quercus pubescens* group has been simplified, but not completely resolved. In fact, the recognition at the specific rank of *taxa* as *Quercus congesta*, *Q. ichnusae*, and *Q. virgiliana*, currently accepted, mainly in local floras in southern Italy, was not supported by genetic results, but a distinction on a geographic basis of the group into two clusters was identified. However, since these two clusters identified revel a clear, but not adequately strong, genetic signal, it can be supposed that the populations sampled belong to a single highly variable pubescent oak *taxon* which is characterized by a large ecological and morphological amplitude. This finding is consistent with morphological and molecular analyses carried out on pubescent oak populations in southeastern Italy (Di Pietro et al. 2016, 2020, 2021), which showed that neither the morphological nor the molecular results supported the existence of more than one pubescent oak species, despite what reported in previous phytosociological studies (Biondi et al. 2004, 2010). Also in the collective group of *Quercus petraea*, differences followed mainly a geographic criterion and, although different subclusters could be distinguished, the elevation at the specific rank for *taxa* as *Q. banatus* and *Q. petraea* subsp. *austrotyrrhenica* has not been supported by the genetic analysis of this study.

In light of these considerations, the need to provide a taxonomic revision that can unify genetically indistinct *taxa* under the same name would be needed. Furthermore, a too divisive classification within the collective group of *Quercus pubescens*, as well as of *Q. petraea*, should be replaced by a more "minimalist" view (Di Pietro et al. 2021).

It is important also considering the role of glacial refugia of the Pleistocene in the southern peninsulas of Iberia, Italy, and Balkans, in order to preserve the genetic pool of the European white oaks as they might provide a unique genetic resource. The analyses of genetic variability and structure are also essential for designing conservation management strategies. In fact, oaks species have implications in forest economy (timber certification), forest management, as well as in nature conservation (Di Pietro et al. 2021). For instance, the *Quercus pubescens* 91AA* priority habitat (eastern white oak woods - Annex I of the Habitats Directive) is one of the forest habitats types under threat of invasion by invasive alien tree species according to the Italian Manual of Interpretation of Habitats of Directive 92/43/EEC (Biondi et al. 2009).

In addition, in a future perspective, a better understanding of genetic mechanisms of variation and adaptation could prove to be useful to adjust conservation measures in response to rapid climatic change. Thus, identifying the potential genetic adaptability of individuals to be used for assisted migration or reintroduction could prove extremely efficient (Aitken and Whitlock 2013; Gonzalez e al. 2013).

The landscape genomics investigates the interaction and connection between adaptive genetic loci on genomes (Storfer et al. 2018) and the aspects of the environment that affect genetic variation and how that variation in turn affects adaptation (Rellstab et al. 2015; Li et al. 2017). These studies can be used to analyse alleles that emerge under particular climatic and habitat circumstances and to understand oaks' reactions to environmental stresses. Identification of populations or individuals that are responding positively to conditions that are expected in the climatic future will help with conservation planning for plant migration or restoration (Rellstab et al. 2016; Martins et al. 2018; Backs and Ashley 2021).

As data about the *Quercus* genus are still lacking in this respect, the need to deepen the current knowledge, using emerging genomic tools and more conventional population genetic analyses, proves necessary in order to better target conservation strategies, which are in any case crucial in view of climate change, even in a genus where unquestionable evolutionary success is well documented and known. Several are the reasons for this great success of oaks' group. According to the interesting treatment of Kremer and Hipp (2020) four main explanations can be identified: the high diversity within populations and species; the rapid migration combined with the ability to adapt to new conditions; the high rates of ecological divergence within clades, combined with convergent solutions to ecological problems across clades; the hybridization and introgression phenomena. Whereas these features are individually shared with other tree genera, the evolutionary success of *Quercus* is indeed due to their combined impacts.

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Supplementary File

Supplementary File 1 Populations of the *Quercus* genus sampled

ID Population	Taxon	Country	Administrative Region (Italy)	Locality	Latitude Coordinates	Longitude Coordinates
AT01	*Quercus pubescens*	Austria		Leopoldsberg, Vienna	48.2750	16.3500
AT02	*Quercus petraea*	Austria		Pfaffenberg, Vienna	48.2611	16.3056
BG03	*Quercus pubescens*	Bulgaria		Kushin village, Pleven district	43.3753	24.5681
HR04	*Quercus pubescens*	Croatia		Mt. Kalnik	46.1311	16.4558
HR05	*Quercus pubescens*	Croatia		Muć	43.7025	16.4253
HR06	*Quercus petraea*	Croatia		Mt. Psunj	45.3223	17.3459
HR07	*Quercus pubescens*	Croatia		Dubrovnik	42.6499	18.0748
FR08	*Quercus pubescens*	France		Donzère Ouest Ligue L.G.V.	44.4475	4.7067
FR09	*Quercus pubescens*	France		Montjoyer Drôme	44.4715	4.8509
FR10	*Quercus pubescens*	France		Donzère Nord est village	44.4431	4.7149
GR11	*Quercus pubescens*	Greece		Pinios Lake	37.9500	21.3833
IT12	*Quercus frainetto*	Italy	Basilicata	Rustico, San Martino d'Agri (PZ)	40.2422	16.0552
IT13	*Quercus robur*	Italy	Basilicata	Oasis Pantano Policoro (MT)	40.1741	16.6998
IT14	*Quercus robur*	Italy	Lazio	Insugherata (La Storta)	42.0073	12.3901
IT15	*Quercus pubescens*	Italy	Lazio	Insugherata (Selva Candida)	41.9547	12.4195
IT16	*Quercus petraea* subsp. *austrotyrrhenica*	Italy	Sicily	Pomieri Wood, Geraci Siculo (PA)	37.8583	14.0581
IT17	*Quercus petraea* subsp. *austrotyrrhenica*	Italy	Calabria	Pollia (Aspromonte), San Luca	38.1460	16.0599
IT18	*Quercus pubescens*	Italy	Veneto	Euganei Hills	45.2745	11.7417
IT19	*Quercus dalechampii*	Italy	Veneto	Euganei Hills	45.3431	11.7617
IT20	*Quercus petraea*	Italy	Veneto	Euganei Hills	45.3173	11.6878
IT21	*Quercus robur*	Italy	Veneto	Euganei Hills	45.3228	11.7409
IT22	*Quercus pubescens*	Italy	Lombardy	One, San Martino (BS)	45.4314	10.6282
IT23	*Quercus pubescens*	Italy	Lazio	Monte San Biagio Plain	41.3641	13.3391
IT24	*Quercus frainetto*	Italy	Lazio	Selvapiana Wood, Amaseno	41.4862	13.3106
IT25	*Quercus virgiliana*	Italy	Sicily	Fitalia Valley (Frazzanò, ME)	38.0689	14.7374
IT26	*Quercus frainetto*	Italy	Lazio	Aurunci	41.3588	13.5272
IT27	*Quercus dalechampii*	Italy	Calabria	Serre, Sant'Angelo SS182 (VV)	38.6265	16.1629
IT28	*Quercus dalechampii*	Italy	Calabria	Aspromonte, SP Palmi Pontevecchio. Croce Mammone (Cirello), Rizziconi (RC)	38.3778	15.9396
IT29	*Quercus congesta*	Italy	Calabria	Aspromonte, SP 3 towards Piani di Carmelia	38.2107	15.9163
CZ30	*Quercus pubescens*	Czech Republic		Milovice	50.3022	14.8739
CZ31	*Quercus petraea*	Czech Republic		Kohoutovice	49.2842	16.7974
CZ32	*Quercus robur*	Czech Republic		Lednice	48.7526	16.8152
RO33	*Quercus banatus*	Romania		Oravița	45.0434	21.7449
RS34	*Quercus frainetto*	Serbia		Tara Mountain	43.9032	19.4838
RS35	*Quercus pubescens*	Serbia		Grgurevci	45.1417	19.6640
RS36	*Quercus petraea*	Serbia		Vrdnik	45.1296	19.8062
RS37	*Quercus dalechampii*	Serbia		Tara Mountain	43.9032	19.4838
RS38	*Quercus dalechampii*	Serbia		Mt. Suvobor	44.1168	20.1588
RS39	*Quercus robur*	Serbia		Mt. Suvobor	44.1168	20.1588
ES40	*Quercus pyrenaica*	Spain		Ranera, Partido de la Sierra en Tobalina, Burgos	42.7186	-3.2837
ES41	*Quercus robur*	Spain		Leioa	43.3253	-2.9708
ES42	*Quercus faginea*	Spain		Zaldiaran	42.7911	-2.7283
ES43	*Quercus petraea*	Spain		Las Machorras	43.1189	-3.5808
ES44	*Quercus pubescens*	Spain		Madotz	42.9350	-1.8792
ES45	*Quercus pubescens*	Spain		Zilbeti	42.9519	-1.4778
IT46	*Quercus petraea*	Italy	Lazio	Mt. Cimini	42.3237	12.1303
IT47	*Quercus petraea*	Italy	Lazio	Tolfa	42.1470	11.9407
IT48	*Quercus ichnusae*	Italy	Sardinia	Senis	39.8159	8.9486
IT49	*Quercus virgiliana*	Italy	Sardinia	Pau	39.7871	8.7892
IT50	*Quercus ichnusae*	Italy	Sardinia	Monte Traessu	40.4686	8.6722
IT51	*Quercus virgiliana*	Italy	Sardinia	Ittiri	40.6138	8.5034
IT52	*Quercus congesta*	Italy	Sardinia	Fonni (Muggiana)	40.1096	9.2490
IT53	*Quercus congesta*	Italy	Sardinia	Fonni (Govossai)	40.1292	9.3024
IT54	*Quercus leptobalanos*	Italy	Sicily	Ficuzza Wood (PA)	37.8841	13.3832
IT55	*Quercus virgiliana*	Italy	Sicily	Marineo (PA)	37.9563	13.4240
IT56	*Quercus congesta*	Italy	Sicily	Etna	37.7095	14.9621
IT57	*Quercus dalechampii*	Italy	Sicily	Etna	37.7063	14.9672
IT58	*Quercus virgiliana*	Italy	Sicily	Etna. Mt. Ceraulo, Mascalucia (CT)	37.5917	15.0448
IT59	*Quercus virgiliana*	Italy	Sicily	Etna. Tre castagni, Via P. Togliatti (CT)	37.6086	15.0724
IT60	*Quercus congesta*	Italy	Sicily	Madonie, between Piano Torre and Piano Zucchi (Collesano, PA)	37.9054	13.9937
IT61	*Quercus congesta*	Italy	Sicily	Valley of Flascio, Nebrodi	37.9416	14.8762
IT62	*Quercus robur*	Italy	Apulia	Lake of Laterza, Tajuri (BA)	40.6259	16.8121
IT63	*Quercus dalechampii*	Italy	Calabria	Serre, Sant'Angelo SS182 crossroads SP Nardodipace (VV)	38.4814	16.3411
IT64	*Quercus pubescens*	Italy	Lazio	Aurunci	41.3588	13.5272
IT65	*Quercus robur*	Italy	Apulia	Lake of Laterza, Tajuri (BA)	41.0926	16.8223
IT66	*Quercus virgiliana*	Italy	Apulia	Lake of Laterza, Tajuri (BA)	41.0926	16.8223
RS67	*Quercus dalechampii*	Serbia		Kopaonik National Park	43.2756	20.8306
IT68	*Quercus pubescens*	Italy	Sardinia	Mt. Zara, Monastir (CA)	39.3775	9.0595
IT69	*Quercus ichnusae*	Italy	Sardinia	S. Antonio Wood, Macomer (NU)	40.2382	8.6979
IT70	*Quercus congesta*	Italy	Sardinia	Sant'Orsola (SS)	40.7462	8.5361
IT71	*Quercus congesta*	Italy	Sardinia	Mt. Rasu, Catena del Marghine, Bono (SS)	40.4278	9.0070
IT72	*Quercus pubescens*	Italy	Basilicata	Laurenzana (PZ)	40.4600	15.9745
IT73	*Quercus petraea*	Italy	Molise	Monte Vairano	41.5500	14.5833
IT75	*Quercus pubescens*	Italy	Molise	Monte Vairano	41.5500	14.5833
IT76	*Quercus pubescens*	Italy	Calabria	Nardodipace	38.4814	16.3411
IT77	*Quercus pubescens*	Italy	Apulia	Bosco del Compare	40.6584	17.8878
IT82	*Quercus pubescens*	Italy	Lazio	Torrita, Laga Mountains (Amatrice, RI)	42.6206	13.2173
IT83	*Quercus pubescens*	Italy	Abruzzo	Barisciano, San Colombo (AQ)	42.3362	13.5901
IT84	*Quercus robur*	Italy	Lombardy	Groane Park, Solaro (MI)	45.6213	9.0943
IT85	*Quercus robur*	Italy	Lombardy	Bosco Fontana, Marmirolo (MN)	45.2046	10.7534
IT86	*Quercus robur*	Italy	Piedmont	Natural Park La Mandria, Venaria Reale (TO)	45.1486	7.5963
IT87	*Quercus pubescens*	Italy	Basilicata	Sasso di Castalda + Satriano	40.5150	15.6849
IT88	*Quercus pubescens*	Italy	Basilicata	Moliterno + Sarconi	40.2535	15.8756
IT89	*Quercus pubescens*	Italy	Basilicata	Fosso Cardone + Catarozzolo	40.1181	16.2214
IT90	*Quercus frainetto*	Italy	Umbria	S.I.C. IT5210077, Collestrada (PG)	43.0947	12.4672
IT91	*Quercus frainetto*	Italy	Calabria	National Park Aspromonte, Platì (RC)	38.2247	16.0454
IT92	*Quercus pubescens*	Italy	Friuli V. Giulia	Basovizza (TS)	45.6283	13.8785
IT93	*Quercus pubescens*	Italy	Marche	Selva of Castelfidardo (AN)	43.4676	13.5918
IT94	*Quercus petraea*	Italy	Umbria	Montecorona, Umbertide (PG)	43.2838	12.3421
IT95	*Quercus petraea*	Italy	Tuscany	Montefalcone Natural Reserve, Castelfranco di Sotto (PI)	43.7354	10.7334
IT96	*Quercus petraea*	Italy	Emilia-Romagna	Parma	44.8298	10.3196
ES85	*Quercus faginea*	Spain		Cordoba	37.9252	-4.8265
MA86	*Quercus faginea*	Morocco		Ahfir	34.9668	-2.0865

Supplementary File 2 Populations of the *Quercus* genus analysed by means of cpDNA

ID Population	Taxon	Country	Administrative Region (Italy)	Locality	Latitude Coordinates	Longitude Coordinates
AT01	*Quercus pubescens*	Austria		Leopoldsberg, Vienna	48.2750	16.3500
AT02	*Quercus petraea*	Austria		Pfaffenberg, Vienna	48.2611	16.3056
BG03	*Quercus pubescens*	Bulgaria		Kushin village, Pleven district	43.3753	24.5681
HR04	*Quercus pubescens*	Croatia		Mt. Kalnik	46.1311	16.4558
HR05	*Quercus pubescens*	Croatia		Muć	43.7025	16.4253
HR06	*Quercus petraea*	Croatia		Mt. Psunj	45.3223	17.3459
HR07	*Quercus pubescens*	Croatia		Dubrovnik	42.6499	18.0748
FR08	*Quercus pubescens*	France		Donzère Ovest Ligue L.G.V.	44.4475	4.7067
FR09	*Quercus pubescens*	France		Montjoyer Drôme	44.4715	4.8509
FR10	*Quercus pubescens*	France		Donzère Nord est village	44.4431	4.7149
GR11	*Quercus pubescens*	Greece		Pinios Lake	37.9500	21.3833
IT12	*Quercus frainetto*	Italy	Basilicata	Rustico, San Martino d'Agri (PZ)	40.2422	16.0552
IT13	*Quercus robur*	Italy	Basilicata	Oasis Pantano Policoro (MT)	40.1741	16.6998
IT14	*Quercus robur*	Italy	Lazio	Insugherata (La Storta)	42.0073	12.3901
IT15	*Quercus pubescens*	Italy	Lazio	Insugherata (Selva Candida)	41.9547	12.4195
IT16	*Quercus petraea* subsp. *austrotyrrhenica*	Italy	Sicily	Pomieri Wood, Geraci Siculo (PA)	37.8583	14.0581
IT17	*Quercus petraea* subsp. *austrotyrrhenica*	Italy	Calabria	Pollia (Aspromonte), San Luca	38.1460	16.0599
IT18	*Quercus pubescens*	Italy	Veneto	Euganei Hills	45.2745	11.7417
IT19	*Quercus dalechampii*	Italy	Veneto	Euganei Hills	45.3431	11.7617
IT20	*Quercus petraea*	Italy	Veneto	Euganei Hills	45.3173	11.6878
IT21	*Quercus robur*	Italy	Veneto	Euganei Hills	45.3228	11.7409
IT22	*Quercus pubescens*	Italy	Lombardy	One, San Martino (BS)	45.4314	10.6282
IT23	*Quercus pubescens*	Italy	Lazio	Monte San Biagio Plain	41.3641	13.3391
IT24	*Quercus frainetto*	Italy	Lazio	Selvapiana Wood, Amaseno	41.4862	13.3106
IT25	*Quercus virgiliana*	Italy	Sicily	Fitalia Valley (Frazzanò, ME)	38.0689	14.7374
IT26	*Quercus frainetto*	Italy	Lazio	Aurunci	41.3588	13.5272
IT27	*Quercus dalechampii*	Italy	Calabria	Serre, Sant'Angelo SS182 (VV)	38.6265	16.1629
IT28	*Quercus dalechampii*	Italy	Calabria	Aspromonte, SP Palmi Pontevecchio. Croce Mammone (Cirello), Rizziconi (RC)	38.3778	15.9396
IT29	*Quercus congesta*	Italy	Calabria	Aspromonte, SP 3 towards Piani di Carmelia	38.2107	15.9163
CZ30	*Quercus pubescens*	Czech Republic		Milovice	50.3022	14.8739
CZ31	*Quercus petraea*	Czech Republic		Kohoutovice	49.2842	16.7974
CZ32	*Quercus robur*	Czech Republic		Lednice	48.7526	16.8152
RO33	*Quercus banatus*	Romania		Oravița	45.0434	21.7449
RS34	*Quercus frainetto*	Serbia		Tara Mountain	43.9032	19.4838
RS35	*Quercus pubescens*	Serbia		Grgurevci	45.1417	19.6640
RS36	*Quercus petraea*	Serbia		Vrdnik	45.1296	19.8062
RS37	*Quercus dalechampii*	Serbia		Tara Mountain	43.9032	19.4838
RS38	*Quercus dalechampii*	Serbia		Mt. Suvobor	44.1168	20.1588
RS39	*Quercus robur*	Serbia		Mt. Suvobor	44.1168	20.1588
ES40	*Quercus pyrenaica*	Spain		Ranera, Partido de la Sierra en Tobalina, Burgos	42.7186	-3.2837
ES41	*Quercus robur*	Spain		Leioa	43.3253	-2.9708
ES42	*Quercus faginea*	Spain		Zaldiaran	42.7911	-2.7283
ES43	*Quercus petraea*	Spain		Las Machorras	43.1189	-3.5808
ES44	*Quercus pubescens*	Spain		Madotz	42.9350	-1.8792
ES45	*Quercus pubescens*	Spain		Zilbeti	42.9519	-1.4778
IT46	*Quercus petraea*	Italy	Lazio	Mt. Cimini	42.3237	12.1303
IT47	*Quercus petraea*	Italy	Lazio	Tolfa	42.1470	11.9407
IT48	*Quercus ichnusae*	Italy	Sardinia	Senis	39.8159	8.9486
IT49	*Quercus virgiliana*	Italy	Sardinia	Pau	39.7871	8.7892
IT50	*Quercus ichnusae*	Italy	Sardinia	Monte Traessu	40.4686	8.6722
IT51	*Quercus virgiliana*	Italy	Sardinia	Ittiri	40.6138	8.5034
IT52	*Quercus congesta*	Italy	Sardinia	Fonni (Muggiana)	40.1096	9.2490
IT53	*Quercus congesta*	Italy	Sardinia	Fonni (Govossai)	40.1292	9.3024
IT54	*Quercus leptobalanos*	Italy	Sicily	Ficuzza Wood (PA)	37.8841	13.3832
IT55	*Quercus virgiliana*	Italy	Sicily	Marineo (PA)	37.9563	13.4240
IT56	*Quercus congesta*	Italy	Sicily	Etna	37.7095	14.9621
IT57	*Quercus dalechampii*	Italy	Sicily	Etna	37.7063	14.9672
IT58	*Quercus virgiliana*	Italy	Sicily	Etna. Mt. Ceraulo, Mascalucia (CT)	37.5917	15.0448
IT59	*Quercus virgiliana*	Italy	Sicily	Etna. Tre castagni, Via P. Togliatti (CT)	37.6086	15.0724
IT60	*Quercus congesta*	Italy	Sicily	Madonie, between Piano Torre and Piano Zucchi (Collesano, PA)	37.9054	13.9937
IT61	*Quercus congesta*	Italy	Sicily	Valley of Flascio, Nebrodi	37.9416	14.8762
IT62	*Quercus robur*	Italy	Apulia	Lake of Laterza, Tajuri (BA)	40.6259	16.8121
IT63	*Quercus dalechampii*	Italy	Calabria	Serre, Sant'Angelo SS182 crossroads SP Nardodipace (VV)	38.4814	16.3411
IT64	*Quercus pubescens*	Italy	Lazio	Aurunci	41.3588	13.5272
IT65	*Quercus robur*	Italy	Apulia	Lake of Laterza, Tajuri (BA)	41.0926	16.8223
IT66	*Quercus virgiliana*	Italy	Apulia	Lake of Laterza, Tajuri (BA)	41.0926	16.8223
RS67	*Quercus dalechampii*	Serbia		Kopaonik National Park	43.2756	20.8306
IT68	*Quercus pubescens*	Italy	Sardinia	Mt. Zara, Monastir (CA)	39.3775	9.0595
IT69	*Quercus ichnusae*	Italy	Sardinia	S. Antonio Wood, Macomer (NU)	40.2382	8.6979
IT70	*Quercus congesta*	Italy	Sardinia	Sant'Orsola (SS)	40.7462	8.5361
IT71	*Quercus congesta*	Italy	Sardinia	Mt. Rasu, Catena del Marghine, Bono (SS)	40.4278	9.0070
IT72	*Quercus pubescens*	Italy	Basilicata	Laurenzana (PZ)	40.4600	15.9745
IT73	*Quercus petraea*	Italy	Molise	Monte Vairano	41.5500	14.5833
IT75	*Quercus pubescens*	Italy	Molise	Monte Vairano	41.5500	14.5833
IT76	*Quercus pubescens*	Italy	Calabria	Nardodipace	38.4814	16.3411
IT77	*Quercus pubescens*	Italy	Apulia	Bosco del Compare	40.6584	17.8878
IT82	*Quercus pubescens*	Italy	Lazio	Torrita, Laga Mountains (Amatrice, RI)	42.6206	13.2173
IT83	*Quercus pubescens*	Italy	Abruzzo	Barisciano, San Colombo (AQ)	42.3362	13.5901
IT84	*Quercus robur*	Italy	Lombardy	Groane Park, Solaro (MI)	45.6213	9.0943
IT85	*Quercus robur*	Italy	Lombardy	Bosco Fontana, Marmirolo (MN)	45.2046	10.7534
IT86	*Quercus robur*	Italy	Piedmont	Natural Park La Mandria, Venaria Reale (TO)	45.1486	7.5963
IT90	*Quercus frainetto*	Italy	Umbria	S.I.C. IT5210077, Collestrada (PG)	43.0947	12.4672
IT91	*Quercus frainetto*	Italy	Calabria	National Park Aspromonte, Platì (RC)	38.2247	16.0454
IT92	*Quercus pubescens*	Italy	Friuli V. Giulia	Basovizza (TS)	45.6283	13.8785
IT93	*Quercus pubescens*	Italy	Marche	Selva of Castelfidardo (AN)	43.4676	13.5918
IT94	*Quercus petraea*	Italy	Umbria	Montecorona, Umbertide (PG)	43.2838	12.3421
IT95	*Quercus petraea*	Italy	Tuscany	Montefalcone Natural Reserve, Castelfranco di Sotto (PI)	43.7354	10.7334
IT96	*Quercus petraea*	Italy	Emilia-Romagna	Parma	44.8298	10.3196
ES85	*Quercus faginea*	Spain		Cordoba	37.9252	-4.8265
MA86	*Quercus faginea*	Morocco		Ahfir	34.9668	-2.0865

Supplementary File 3 Samples of the *Quercus* genus from the current study and from GenBank analysed by means of cpDNA

No. Sequence	Taxon	Section / Lineage	Region	Origin	Locality	Code	trnH-psbA (accession # Genbank)	trnK-matK (accession # Genbank)	Plastid haplotype	References
1	Quercus infectoria, Q. kotschyana, Q. cedrorum	West Eurasian Quercus	Middle East	Lebanon	Various	H01Lib	MN955848	MT041757	H01	Douaihy B, Saliba C, Stephan J, Simeone MC, Cardoni S, Farhat P, Bou Dagher, Kharrat M (2020) Tracking diversity and evolutionary pathways of Lebanese oak taxa through plastome analyses. Botany Letters 167:315-330. DOI: 10.1080/23818107.2020.1765197
2	Hybrid unknown	West Eurasian Quercus	Middle East	Lebanon	Various	H02Lib	MN955825	MT041734	H02	
3	Hybrid unknown	West Eurasian Quercus	Middle East	Lebanon	Various	H03Lib	MN955826	MT41735	H03	
4	Quercus infectoria, Q. kotschyana	West Eurasian Quercus	Middle East	Lebanon	Various	H04Lib	MN955828	MT041737	H04	
5	Quercus infectoria	West Eurasian Quercus	Middle East	Lebanon	Various	H05Lib	MN955839	MT041748	H05	
6	Quercus macranthera, Q. petraea subsp. iberica, Q. robur subsp. imeretina	West Eurasian Quercus	Caucasus	Georgia	Manglisi	H01Geo	LT718052	LT718145	H04	Ekhvaia J, Simeone MC, Silakadze N, Abdaladze O (2018) Morphological diversity and phylogeography of the Georgian durmast oak (Q. petraea subsp. iberica) and related Caucasian oak species in Georgia (South Caucasus). Tree Genetics and Genomes 14:17. DOI: 10.1007/s11295-018-1232-6
7	Quercus petraea subsp. iberica, Q. robur subsp. imeretina	West Eurasian Quercus	Caucasus	Georgia	Tsageri	H02Geo	LT718016	LT718109	H06	
8	Quercus petraea subsp. iberica	West Eurasian Quercus	Caucasus	Georgia	Tskneti	H03Geo	LT718075	LT718168	H07	
9	Quercus petraea subsp. iberica, Q. robur subsp. pedunculiflora	West Eurasian Quercus	Caucasus	Georgia	Gombori	H04Geo	LT718083	LT718176	H08	
10	Quercus hartwissiana, Q. petraea spp., Q. robur spp.	West Eurasian Quercus	Caucasus	Georgia	Etseri	H05Geo	LT718014	LT718107	H04	
11	Quercus pontica	West Eurasian Ponticae	Caucasus	Georgia	Keda	H06Geo	LT718044	LT718137	H09	
12	Quercus pontica	West Eurasian Ponticae	Caucasus	Georgia	Keda	H07Geo	LT718046	LT718139	H10	
13	Quercus hartwissiana	West Eurasian Quercus	Caucasus	Georgia	Keda	H08Geo	LT718012	LT718105	H11	
14	Quercus petraea subsp. petraea	West Eurasian Quercus	C Europe	Italy	C Italy (Latium)	QPETLA	HE591284	HE583641	H12	Simeone MC, Piredda R, Papini A, Vessella F, Schirone B (2013) Application of plastid and nuclear markers to DNA barcoding of Euro Mediterranean oaks (Quercus, Fagaceae): problems, prospects and phylogenetic implications. Botanical Journal of the Linnean Society 172:478-499. DOI 10.1111/boj.12059.
15	Quercus petraea subsp. huguetiana	West Eurasian Quercus	W Europe	Spain	n.d.	QPETES	HE591307	HE583664	H13	
16	Quercus petraea subsp. petraea	West Eurasian Quercus	E Europe	Greece	n.d.	QPETGR	HE591314	HE583671	H12	
17	Quercus petraea subsp. petraea	West Eurasian Quercus	C Europe	Italy	N Italy (Piedmont)	QPETPI	HE591282	HE583639	H06	
18	Quercus petraea subsp. sessiliflora (syn. ssp. petraea)	West Eurasian Quercus	E Europe	Bulgaria	Rila Mts.	QPETBG	HE591283	HE583640	H14	
19	Quercus petraea subsp. dshorochensis (syn. subsp. iberica)	West Eurasian Quercus	Caucasus	Georgia	n.d.	QPETGE	HE591323	HE583723	H06	
20	Quercus petraea subsp. petraea	West Eurasian Quercus	C Europe	Italy	S Italy (Sicily)	QPETSI	HE591274	HE583631	H15	
21	Quercus polycarpa (syn. Quercus petraea subsp. iberica)	West Eurasian Quercus	E Europe	Romania	n.d.	QPOLRO	HE591324	HE583724	H06	
22	Quercus robur subsp. robur	West Eurasian Quercus	W Europe	Spain	n.d.	QROBES	HE591311	HE583668	H13	
23	Quercus robur subsp. pedunculiflora	West Eurasian Quercus	E Europe	Ukraine	n.d.	QROBUA	HE591312	HE583669	H16	
24	Quercus robur subsp. pedunculiflora	West Eurasian Quercus	E Europe	Bulgaria	Rila Mts.	QROBBG	HE591285	HE583642	H06	
25	Quercus robur subsp. robur	West Eurasian Quercus	C Europe	England	Romsey	QROBUK	HE591287	HE583644	H13	
26	Quercus haas (syn. Quercus robur subsp. robur)	West Eurasian Quercus	Middle East	Turkey	n.d.	QHAATR	HE591325	HE583725	H17	
27	Quercus frainetto	West Eurasian Quercus	C Europe	Italy	C Italy (Latium)	QFRALA	HE591272	HE583629	H04	
28	Quercus frainetto	West Eurasian Quercus	E Europe	Greece	n.d.	QFRAGR	HE591313	HE583670	H18	
29	Quercus frainetto	West Eurasian Quercus	C Europe	Italy	SE Italy (Apulia)	QFRAAP	HE591271	HE583628	H15	
30	Quercus frainetto	West Eurasian Quercus	E Europe	Bulgaria	Rila Mts.	QFRABG	HE591279	HE583636	H12	
31	Quercus pubescens subsp. pubescens	West Eurasian Quercus	C Europe	Italy	C Italy (Latium)	QPUBLA	HE591288	HE583645	H19	
32	Quercus pubescens subsp. pubescens	West Eurasian Quercus	C Europe	Croatia	n.d.	QPUBHR	HE591308	HE583665	H04	
33	Quercus pubescens subsp. anatolica	West Eurasian Quercus	E Europe	Greece	n.d.	QPUBGR	HE591309	HE583666	H13	
34	Quercus pubescens subsp. subpyrenaica	West Eurasian Quercus	W Europe	Spain	n.d.	QPUBES	HE591277	HE583634	H20	
35	Quercus congesta	West Eurasian Quercus	C Europe	Italy	Sardinia	QCONSA	HE591289	HE583646	H21	
36	Quercus congesta	West Eurasian Quercus	C Europe	Italy	Sicily	QCONSI	HE591276	HE583633	H15	
37	Quercus iberica	West Eurasian Quercus	Caucasus	Armenia	Ohrid	QIBEAM	HE591280	HE583637	H06	
38	Quercus iberica	West Eurasian Quercus	Middle East	Turkey	n.d.	QIBETR	HE591327	HE583727	H16	
39	Quercus macranthera	West Eurasian Quercus	Caucasus	Armenia	Ohrid	QMACAM	HE591281	HE583638	H06	
40	Quercus macranthera	West Eurasian Quercus	Middle East	Turkey	n.d.	QMACTR	HE591328	HE583728	H22	
41	Quercus pyrenaica	West Eurasian Quercus	W Europe	Spain	n.d.	QPYRES	HE591310	HE583667	H15	
42	Quercus canariensis	West Eurasian Quercus	N Africa	Algeria	Tlemcen	QCANDZ	HE591268	HE583625	H13	
43	Quercus canariensis	West Eurasian Quercus	W Europe	Spain	n.d.	QCANES	HE591329	HE583729	H13	
44	Quercus faginea	West Eurasian Quercus	N Africa	Algeria	Tlemcen	QFAGDZ	HE591269	HE583626	H13	
45	Quercus faginea	West Eurasian Quercus	W Europe	Portugal	Faro	QFAGPT	HE591270	HE583627	H13	
46	Quercus lusitanica	West Eurasian Quercus	W Europe	Portugal	n.d.	QLUSPT	HE591303	HE583660	H13	
47	Quercus hartwissiana	West Eurasian Quercus	E Europe	Bulgaria	Rila Mts.	QHARBG	HE591273	HE583630	H04	
48	Quercus hartwissiana	West Eurasian Quercus	E Europe	Bulgaria	n.d.	QHARVA	HE611278	HE611283	H13	
49	Quercus infectoria	West Eurasian Quercus	Middle East	Cyprus	n.d.	QINFCY	HE591304	HE583661	H13	
50	Quercus infectoria	West Eurasian Quercus	Middle East	Turkey	n.d.	QINFTR	HE591305	HE583662	H23	
51	Quercus infectoria	West Eurasian Quercus	Middle East	Lebanon	n.d.	QINFLE	HE591306	HE583663	H07	
52	Quercus vulcanica	West Eurasian Quercus	Middle East	Turkey	Isparta	QVULTR	HE591278	HE583635	H24	
53	Quercus protoroburoides	West Eurasian Quercus	E Europe	Bulgaria	Rila Mts.	QPROBG	HE591275	HE583632	H13	
54	Quercus mongolica	East Eurasian Quercus	Asia	Korea	n.d.	QMONKR	HE585134	HE585139	H28	
55	Quercus serrata	East Eurasian Quercus	Asia	Korea	n.d.	QSERKR	HE585135	HE585140	H29	
56	Quercus aliena	East Eurasian Quercus	Asia	Korea	n.d.	QALIKR	HE585133	HE585141	H07	
57	Quercus fabri	East Eurasian Quercus	Asia	China	n.d.	QFABCN	HE591337	HE583737	H13	
58	Quercus agrifolia	Lobatae	America	WN America	California	QAGRCA	LM652856	LM652873	H30	Simeone MC, Grimm GW, Papini A, Vessella F, Cardoni S, Tordoni E, Piredda R, Franc A, Denk T (2016) Plastome data reveal multiple geographic origins of Quercus group Ilex. PeerJ. 4:e1897
59	Quercus alba	American Quercus	America	N America	Illinois	QALBIL	LM652853	LM652870	H31	
60	Quercus berberidifolia	American Quercus	America	WN America	California	QBERC1	LM652858	LM652883	H32	
61	Quercus berberidifolia	American Quercus	America	WN America	California	QBERC2	LM652859	LM652892	H33	
62	Quercus bicolor	American Quercus	America	N America	Michigan	QBICMI	LM652854	LM652871	H34	
63	Quercus cedrosensis	Protobalanus	America	C America	Mexico	QCEDMX	LM652795	LM652925	H39	
64	Quercus chrysolepis	Protobalanus	America	WN America	California	QCHRC1	HE611280	HE611285	H40	
65	Quercus chrysolepis	Protobalanus	America	WN America	California	QCHRC2	LM652836	LM652884	H41	
66	Quercus chrysolepis	Protobalanus	America	WN America	California	QCHRC3	LM652837	LM652885	H42	
67	Quercus crassipes	Lobatae	America	C America	Mexico	QCRAMX	LM652855	LM652872	H44	
68	Quercus dentata	East Eurasian Quercus	Asia	Japan	n.d.	QDENJP	HE591335	HE583735	H13	
69	Quercus ellipsoidalis	Lobatae	America	EN America	Michigan	QELLMI	LM652860	LM652874	H45	
70	Quercus emoryi	Lobatae	America	WN America	Arizona	QEMOAR	LM652799	LM652940	H46	
71	Quercus fusiformis	Virentes	America	WN America	n.d.	QFUSUS	LM652861	LM652881	H47	
72	Quercus geminata	Virentes	America	EN America	Georgia	QGEMGE	LM652804	LM652942	H35	
73	Quercus griffithii	East Eurasian Quercus	Asia	China	n.d.	QGRICN	HE591336	HE583736	H36	
74	Quercus iberica	West Eurasian Quercus	Middle East	Iran	n.d.	QIBEIR	HE591326	HE583726	H25	
75	Quercus incana	Lobatae	America	EN America	Alabama	QINCAL	LM652808	LM652957	H48	
76	Quercus macrocarpa	American Quercus	America	EN America	n.d.	QMACUS	HE611279	HE611284	H37	
77	Quercus minima	Virentes	America	EN America	Georgia	QMINGE	LM652805	LM652958	H49	
78	Quercus myrtifolia	Lobatae	America	EN America	Florida	QMYRFL	LM652798	LM652959	H50	
79	Quercus oleoides	Virentes	America	C America	Mexico	QOLEMX	LM652797	LM652960	H51	
80	Quercus palmeri	Protobalanus	America	WN America	California	QPALC1	LM652800	LM652962	H52	
81	Quercus palmeri	Protobalanus	America	WN America	California	QPALC2	LM652830	LM652906	H53	
82	Quercus pontica	West Eurasian Ponticae	Caucasus	Georgia	n.d.	QPONND	HE591330	HE583730	H26	
83	Quercus pontica	West Eurasian Ponticae	Middle East	Turkey	NE Turkey	QPONTR	LM652862	LM652878	H27	
84	Quercus pyrenaica	West Eurasian Quercus	C Europe	France	S France	QPYRFR	HE591339	HE612111	H13	
85	Quercus robur	West Eurasian Quercus	C Europe	Italy	C Italy (Latium)	QROBLA	HE591286	HE583643	H12	
86	Quercus rubra	Lobatae	America	EN America	n.d.	QRUBU1	HE611277	HE611282	H54	
87	Quercus rubra	Lobatae	America	EN America	n.d.	QRUBU2	LM652793	LM652963	H55	
88	Quercus sadleriana	North American Ponticae	America	SW America	California	QSADCA	LM652803	LM652964	H43	
89	Quercus tomentella	Protobalanus	America	WN America	California	QTOMC1	LM652832	LM652893	H56	
90	Quercus tomentella	Protobalanus	America	WN America	California	QTOMC2	LM652833	LM652867	H57	
91	Quercus vacciniifolia	Protobalanus	America	WN America	California	QVACCA	LM652852	LM652904	H38	
92	Quercus vacciniifolia	Protobalanus	America	WN America	Washington	QVACWA	LM652796	LM652966	H58	
93	Quercus virginiana	Virentes	America	EN America	Florida	QVIRFL	LM652801	LM652968	H49	
94	Quercus virginiana	Virentes	America	EN America	n.d.	QVIRU1	LM652802	LM652967	H51	
95	Quercus virginiana	Virentes	America	EN America	n.d.	QVIRU2	LM652834	LM652905	H35	

96	Quercus banatus, Q. dalechampii, Q. frainetto, Q. iberica, Q. petraea, Q. pubescens, Q. robur	West Eurasian Quercus	C and E Europe, Georgia	Austria; Croatia; Czech Republic; France (Rhône-Alpes); Georgia; Italy (N and S); Romania	Various	Qeu01			H06	Current study
97	Quercus dalechampii, Q. frainetto, Q. petraea, Q. petraea subsp. austrotyrrhenica, Q. pubescens, Q. robur, Q. virgiliana	West Eurasian Quercus	C and E Europe	Bulgaria; Croatia; Greece; Italy (C and S); Serbia	Various	Qeu02			H12	
98	Quercus congesta, Q. dalechampii, Q. faginea, Q. petraea, Q. pubescens, Q. robur, Q. virgiliana	West Eurasian Quercus	C and W Europe	Italy (N and C); Spain (N)	Various	Qeu03			H15	
99	Quercus faginea, Q. frainetto, Q. petraea, Q. pubescens, Q. pyrenaica, Q. robur	West Eurasian Quercus	C and W Europe, Morocco	Bulgaria; Italy (C and S); Morocco; Spain (N and Andalucia)	Various	Qeu04			H13	
100	Quercus pubescens	West Eurasian Quercus	C Europe	Croatia	Croatia	Qeu05			H59	
101	Quercus congesta, Q. frainetto, Q. hartwissiana, Q. iberica, Q. leptobalanos, Q. petraea subsp. austrotyrrhenica, Q. pubescens, Q. robur, Q. virgiliana	West Eurasian Quercus	Italy, Georgia	Italy (N and C, Sicily), Georgia	Various	Qeu06			H04	
102	Quercus congesta, Q. dalechampii, Q. ichnusae, Q. virgiliana	West Eurasian Quercus	C Europe	Italy	Sardinia, Calabria	Qeu07			H21	
103	Quercus congesta, Q. dalechampii, Q. iberica	West Eurasian Quercus	Italy, Georgia	Italy (Calabria, Sicily), Georgia	Various	Qeu08			H07	
104	Quercus congesta, Q. ichnusae, Q. virgiliana	West Eurasian Quercus	C Europe	Italy	Sicily	Qeu09			H60	
105	Quercus ichnusae	West Eurasian Quercus	C Europe	Italy	Sardinia	Qeu10			H61	
106	Quercus virgiliana	West Eurasian Quercus	C Europe	Italy	Sardinia	Qeu11			H62	
107	Quercus pubescens	West Eurasian Quercus	C Europe	Italy	Apulia	Qeu12			H14	
108	Quercus faginea	West Eurasian Quercus	W Europe	Spain	Andalucia	Qeu13			H63	
109	Quercus faginea	West Eurasian Quercus	W Europe	Spain	Andalucia	Qeu14			H64	
110	Quercus alnifolia	Ilex/ WAHEA	Middle East	Cyprus	n.d.	WAHEA	LM652764	LM652922	H68	Simeone MC, Grimm GW, Papini A, Vessella F, Cardoni S, Tordoni E, Piredda R, Franc A, Denk T (2016) Plastome data reveal multiple geographic origins of Quercus group Ilex. PeerJ. 4:e1897
111	Quercus baroni	Ilex/E Asian	Asia	China	n.d.	BARONI	LM652806	LM652924	H69	
112	Quercus ilex	Ilex/ West-Med	Asia	Spain	n.d.	ILEMED	LM652780	LM652953	H66	
113	Quercus cerris	Cerris / Cerris-Ilex	C Europe	Italy	n.d.	CERRIS	HE591247	HE591292	H65	
114	Quercus coccifera	Ilex/ West-Med	N Africa	Libya	n.d.	COCMED	LM652767	LM652933	H67	
115	Quercus acuta	Cyclobalanopsis	Asia	China	n.d.	ACUTA	LM652857	LM652886	H70	
116	Notholithocarpus densiflorus		America	America	n.d.	Notholicarpus	LM652863	LM652879	H71	
117	Quercus stewardiana	East Eurasian Quercus	Asia	China	n.d.	Qch01	KX837076	KX837490		Yang J, Guo YF, Chen XD, Zhang X, Ju MM, Bai GQ, Liu ZL, Zhao GF (2020) Framework Phylogeny, Evolution and Complex Diversification of Chinese Oaks. Plants 9(8):1024. DOI: 10.3390/plants9081024
118	Quercus stewardiana, Q. mongolica, Q. wutaishanica, Q. serrata, Q. fabri, Q. aliena	East Eurasian Quercus	Asia	China	n.d.	Qch02	KX837075	KX837489		
119	Quercus yunnanensis, Q. griffithii, Q. mongolica, Q. serrata, Q. dentata, Q. aliena	East Eurasian Quercus	Asia	China	n.d.	Qch03	KX837073	KX837487		
120	Quercus griffithii	East Eurasian Quercus	Asia	China	n.d.	Qch04	KX837067	KX837481		
121	Quercus mongolica, Q. dentata	East Eurasian Quercus	Asia	China	n.d.	Qch05	KX837064	KX837478		
122	Quercus wutaishanica	East Eurasian Quercus	Asia	China	n.d.	Qch06	KX837052	KX837468		
123	Quercus wutaishanica, Q. serrata	East Eurasian Quercus	Asia	China	n.d.	Qch07	KX837049	KX837465		
124	Quercus dentata	East Eurasian Quercus	Asia	China	n.d.	Qch08	KX837017	KX837435		
125	Quercus dentata	East Eurasian Quercus	Asia	China	n.d.	Qch09	KX837018	KX837436		

Supplementary File 4 Populations of the *Quercus* genus analysed by means of nuclear SSRs

No. Population	ID Population	No. of samples	Taxon	Country	Administrative Region (Italy)	Locality	Latitude Coordinates	Longitude Coordinates
1	**IT18**	20	*Quercus pubescens*	Italy	Veneto	Euganei Hills	45.2745	11.7417
2	**IT22**	20	*Quercus pubescens*	Italy	Lombardy	One, San Martino (BS)	45.4314	10.6282
3	**IT15**	20	*Quercus pubescens*	Italy	Lazio	Insugherata (Selva Candida)	41.9547	12.4195
4	**IT23**	20	*Quercus pubescens*	Italy	Lazio	Monte San Biagio Plain	41.3641	13.3391
5	**IT64**	20	*Quercus pubescens*	Italy	Lazio	Aurunci	41.3588	13.5272
6	**IT82**	7	*Quercus pubescens*	Italy	Lazio	Torrita, Laga Mountains (Amatrice, RI)	42.6206	13.2173
7	**IT72**	10	*Quercus pubescens*	Italy	Basilicata	Laurenzana (PZ)	40.4600	15.9745
8	**IT87**	20	*Quercus pubescens*	Italy	Basilicata	Sasso di Castalda + Satriano	40.5150	15.6849
9	**IT88**	20	*Quercus pubescens*	Italy	Basilicata	Moliterno + Sarconi	40.2535	15.8756
10	**IT89**	20	*Quercus pubescens*	Italy	Basilicata	Fosso Cardone + Catarozzolo	40.1181	16.2214
11	**IT66**	8	*Quercus virgiliana*	Italy	Apulia	Lake of Laterza, Tajuri (BA)	41.0926	16.8223
12	**IT48**	20	*Quercus ichnusae*	Italy	Sardinia	Senis	39.8159	8.9486
13	**IT49**	19	*Quercus virgiliana*	Italy	Sardinia	Pau	39.7871	8.7892
14	**IT50**	20	*Quercus ichnusae*	Italy	Sardinia	Monte Traessu	40.4686	8.6722
15	**IT51**	20	*Quercus virgiliana*	Italy	Sardinia	Ittiri	40.6138	8.5034
16	**IT52**	20	*Quercus congesta*	Italy	Sardinia	Fonni (Muggiana)	40.1096	9.2490
17	**IT53**	20	*Quercus congesta*	Italy	Sardinia	Fonni (Govossai)	40.1292	9.3024
18	**ES44**	20	*Quercus pubescens*	Spain		Madotz	42.9350	-1.8792
19	**ES45**	20	*Quercus pubescens*	Spain		Zilbeti	42.9519	-1.4778
20	**FR08**	20	*Quercus pubescens*	France		Donzère Ouest Ligue L.G.V.	44.4475	4.7067
21	**FR09**	20	*Quercus pubescens*	France		Montjoyer Drôme	44.4715	4.8509
22	**FR10**	20	*Quercus pubescens*	France		Donzère Nord est village	44.4431	4.7149
23	**AT01**	20	*Quercus pubescens*	Austria		Leopoldsberg, Vienna	48.2750	16.3500
24	**BG03**	10	*Quercus pubescens*	Bulgaria		Kushin village, Pleven district	43.3753	24.5681
25	**HR04**	20	*Quercus pubescens*	Croatia		Mt. Kalnik	46.1311	16.4558
26	**HR05**	20	*Quercus pubescens*	Croatia		Muć	43.7025	16.4253
27	**HR07**	20	*Quercus pubescens*	Croatia		Dubrovnik	42.6499	18.0748
28	**GR11**	20	*Quercus pubescens*	Greece		Pinios Lake	37.9500	21.3833
29	**CZ30**	20	*Quercus pubescens*	Czech Republic		Milovice	50.3022	14.8739
30	**RS35**	20	*Quercus pubescens*	Serbia		Grgurevci	45.1417	19.6640
31	**IT19**	20	*Quercus dalechampii*	Italy	Veneto	Euganei Hills	45.3431	11.7617
32	**IT20**	20	*Quercus petraea*	Italy	Veneto	Euganei Hills	45.3173	11.6878
33	**IT46**	20	*Quercus petraea*	Italy	Lazio	Mt. Cimini	42.3237	12.1303
34	**IT47**	20	*Quercus petraea*	Italy	Lazio	Tolfa	42.1470	11.9407
35	**IT17**	20	*Quercus petraea* subsp. *austrotyrrhenica*	Italy	Calabria	Pollia (Aspromonte), San Luca	38.1460	16.0599
36	**IT16**	20	*Quercus petraea* subsp. *austrotyrrhenica*	Italy	Sicily	Pomieri Wood, Geraci Siculo (PA)	37.8583	14.0581
37	**ES43**	20	*Quercus petraea*	Spain		Las Machorras	43.1189	-3.5808
38	**AT02**	20	*Quercus petraea*	Austria		Pfaffenberg, Vienna	48.2611	16.3056
39	**HR06**	20	*Quercus petraea*	Croatia		Mt. Psunj	45.3223	17.3459
40	**CZ31**	20	*Quercus petraea*	Czech Republic		Kohoutovice	49.2842	16.7974
41	**RO33**	20	*Quercus banatus*	Romania		Oravița	45.0434	21.7449
42	**RS36**	20	*Quercus petraea*	Serbia		Vrdnik	45.1296	19.8062
43	**RS37**	20	*Quercus dalechampii*	Serbia		Tara Mountain	43.9032	19.4838
44	**RS38**	20	*Quercus dalechampii*	Serbia		Mt. Suvobor	44.1168	20.1588
45	**RS67**	14	*Quercus dalechampii*	Serbia		Kopaonik National Park	43.2756	20.8306
46	**ES41**	20	*Quercus robur*	Spain		Leioa	43.3253	-2.9708
47	**IT21**	10	*Quercus robur*	Italy	Veneto	Euganei Hills	45.3228	11.7409
48	**IT14**	20	*Quercus robur*	Italy	Lazio	Insugherata (La Storta)	42.0073	12.3901
49	**IT13**	20	*Quercus robur*	Italy	Basilicata	Oasis Pantano Policoro (MT)	40.1741	16.6998
50	**IT62**	4	*Quercus robur*	Italy	Apulia	Lake of Laterza, Tajuri (BA)	40.6259	16.8121
51	**CZ32**	20	*Quercus robur*	Czech Republic		Lednice	48.7526	16.8152
52	**RS39**	10	*Quercus robur*	Serbia		Mt. Suvobor	44.1168	20.1588
53	**IT24**	20	*Quercus frainetto*	Italy	Lazio	Selvapiana Wood, Amaseno	41.4862	13.3106
54	**IT26**	20	*Quercus frainetto*	Italy	Lazio	Aurunci	41.3588	13.5272
55	**IT12**	20	*Quercus frainetto*	Italy	Basilicata	Rustico, San Martino d’Agri (PZ)	40.2422	16.0552
56	**RS34**	20	*Quercus frainetto*	Serbia		Tara Mountain	43.9032	19.4838
57	**ES40**	20	*Quercus pyrenaica*	Spain		Ranera, Partido de la Sierra en Tobalina, Burgos	42.7186	-3.2837
58	**ES42**	20	*Quercus faginea*	Spain		Zaldiaran	42.7911	-2.7283

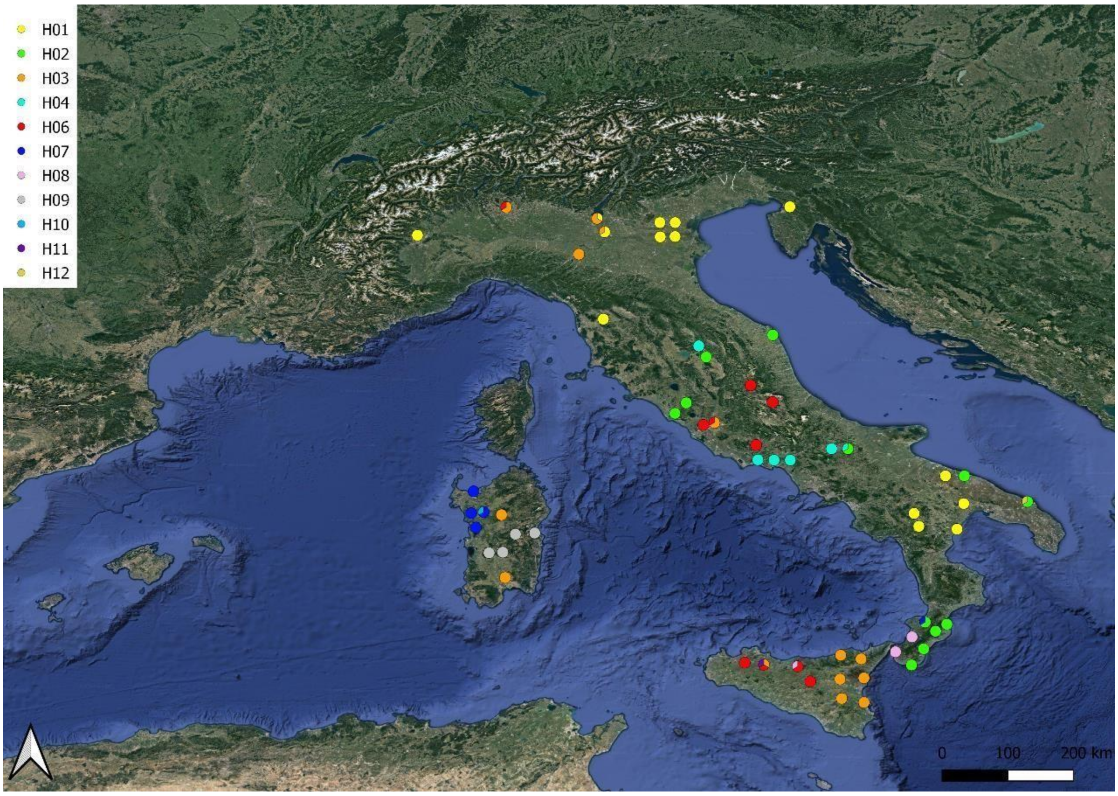
Supplementary File 5 Haplotypes of cpDNA (plastid loci: *trn*H-*psb*A and *trn*K-*mat*K) found in the 270 samples of *Quercus* analysed

Sample N	**ID Polulation**	**Tree**	**Taxon**	**Country**	**Administrative Region (Italy)**	**Locality**	**trnK-matK haplotype**	**trnH-psbA haplotype**	**Plastid haplotype**
1	RO33	1	*Quercus banatus*	Romania		Oravița	K01	T01	H01
2	RO33	2	*Quercus banatus*	Romania		Oravița	K01	T01	H01
3	RO33	3	*Quercus banatus*	Romania		Oravița	K01	T01	H01
4	AT02	28	*Quercus petraea*	Austria		Pfaffenberg, Vienna	K01	T01	H01
5	AT02	29	*Quercus petraea*	Austria		Pfaffenberg, Vienna	K01	T01	H01
6	AT02	30	*Quercus petraea*	Austria		Pfaffenberg, Vienna	K01	T01	H01
7	CZ31	1	*Quercus petraea*	Czech Republic		Kohoutovice	K01	T01	H01
8	CZ31	2	*Quercus petraea*	Czech Republic		Kohoutovice	K01	T01	H01
9	CZ31	3	*Quercus petraea*	Czech Republic		Kohoutovice	K01	T01	H01
10	RS37	1	*Quercus dalechampii*	Serbia		Tara Mountain	K01	T02	H02
11	RS37	2	*Quercus dalechampii*	Serbia		Tara Mountain	K01	T02	H02
12	RS37	3	*Quercus dalechampii*	Serbia		Tara Mountain	K01	T02	H02
13	ES43	1	*Quercus petraea*	Spain		Las Machorras	K02	T03	H03
14	ES43	2	*Quercus petraea*	Spain		Las Machorras	K02	T03	H03
15	ES43	3	*Quercus petraea*	Spain		Las Machorras	K02	T03	H03
16	IT47	2	*Quercus petraea*	Italy	Lazio	Tolfa	K01	T02	H02
17	IT47	3	*Quercus petraea*	Italy	Lazio	Tolfa	K01	T02	H02
18	IT47	4	*Quercus petraea*	Italy	Lazio	Tolfa	K01	T02	H02
19	FR08	8	*Quercus pubescens*	France		Donzère Ovest Ligue L.G.V.	K01	T01	H01
20	FR08	9	*Quercus pubescens*	France		Donzère Ovest Ligue L.G.V.	K01	T01	H01
21	FR08	11	*Quercus pubescens*	France		Donzère Ovest Ligue L.G.V.	K01	T01	H01
22	BG03	1	*Quercus pubescens*	Bulgaria		Kushin village, Pleven district	K01	T02	H02
23	BG03	2	*Quercus pubescens*	Bulgaria		Kushin village, Pleven district	K02	T04	H04
24	BG03	10	*Quercus pubescens*	Bulgaria		Kushin village, Pleven district	K01	T02	H02
25	HR04	1	*Quercus pubescens*	Croatia		Mt. Kalnik	K01	T01	H01
26	HR04	2	*Quercus pubescens*	Croatia		Mt. Kalnik	K01	T01	H01
27	HR04	3	*Quercus pubescens*	Croatia		Mt. Kalnik	K01	T01	H01
28	HR05	1	*Quercus pubescens*	Croatia		Muć	K03	T01	H05
29	HR05	2	*Quercus pubescens*	Croatia		Muć	K03	T01	H05
30	HR05	3	*Quercus pubescens*	Croatia		Muć	K03	T01	H05
31	HR06	1	*Quercus petraea*	Croatia		Mt. Psunj	K01	T01	H01
32	HR06	2	*Quercus petraea*	Croatia		Mt. Psunj	K01	T01	H01
33	HR06	3	*Quercus petraea*	Croatia		Mt. Psunj	K01	T01	H01
34	HR07	1	*Quercus pubescens*	Croatia		Dubrovnik	K01	T02	H02
35	HR07	2	*Quercus pubescens*	Croatia		Dubrovnik	K01	T02	H02
36	HR07	3	*Quercus pubescens*	Croatia		Dubrovnik	K01	T02	H02
37	FR09	1	*Quercus pubescens*	France		Montjoyer Drôme	K01	T01	H01
38	FR09	2	*Quercus pubescens*	France		Montjoyer Drôme	K01	T01	H01
39	FR09	3	*Quercus pubescens*	France		Montjoyer Drôme	K01	T01	H01
40	FR10	1	*Quercus pubescens*	France		Donzère Nord est village	K01	T01	H01
41	FR10	3	*Quercus pubescens*	France		Donzère Nord est village	K01	T01	H01
42	FR10	4	*Quercus pubescens*	France		Donzère Nord est village	K01	T01	H01
43	GR11	1	*Quercus pubescens*	Greece		Pinios Lake	K01	T02	H02
44	GR11	2	*Quercus pubescens*	Greece		Pinios Lake	K01	T02	H02
45	GR11	3	*Quercus pubescens*	Greece		Pinios Lake	K01	T02	H02
46	IT12	1	*Quercus frainetto*	Italy	Basilicata	Rustico, San Martino d'Agri (PZ)	K01	T01	H01
47	IT12	2	*Quercus frainetto*	Italy	Basilicata	Rustico, San Martino d'Agri (PZ)	K01	T01	H01
48	IT12	3	*Quercus frainetto*	Italy	Basilicata	Rustico, San Martino d'Agri (PZ)	K01	T01	H01
49	IT13	1	*Quercus robur*	Italy	Basilicata	Oasis Pantano Policoro (MT)	K01	T01	H01
50	IT13	2	*Quercus robur*	Italy	Basilicata	Oasis Pantano Policoro (MT)	K01	T01	H01
51	IT13	3	*Quercus robur*	Italy	Basilicata	Oasis Pantano Policoro (MT)	K01	T01	H01
52	IT14	1	*Quercus robur*	Italy	Lazio	Insugherata (La Storta)	K02	T01	H06
53	IT14	2	*Quercus robur*	Italy	Lazio	Insugherata (La Storta)	K02	T03	H03
54	IT14	3	*Quercus robur*	Italy	Lazio	Insugherata (La Storta)	K02	T03	H03
55	IT15	2	*Quercus pubescens*	Italy	Lazio	Insugherata (Selva Candida)	K02	T01	H06
56	IT15	3	*Quercus pubescens*	Italy	Lazio	Insugherata (Selva Candida)	K02	T01	H06
57	IT15	4	*Quercus pubescens*	Italy	Lazio	Insugherata (Selva Candida)	K02	T01	H06
58	IT16	1	*Quercus petraea* subsp. *austrotyrrhenica*	Italy	Sicily	Pomieri Wood, Geraci Siculo (PA)	K02	T01	H06
59	IT16	2	*Quercus petraea* subsp. *austrotyrrhenica*	Italy	Sicily	Pomieri Wood, Geraci Siculo (PA)	K02	T01	H06
60	IT16	3	*Quercus petraea* subsp. *austrotyrrhenica*	Italy	Sicily	Pomieri Wood, Geraci Siculo (PA)	K02	T01	H06
61	IT17	1	*Quercus petraea* subsp. *austrotyrrhenica*	Italy	Calabria	Pollia (Aspromonte), San Luca	K01	T02	H02
62	IT17	2	*Quercus petraea* subsp. *austrotyrrhenica*	Italy	Calabria	Pollia (Aspromonte), San Luca	K01	T02	H02
63	IT17	3	*Quercus petraea* subsp. *austrotyrrhenica*	Italy	Calabria	Pollia (Aspromonte), San Luca	K01	T02	H02
64	IT18	12	*Quercus pubescens*	Italy	Veneto	Euganei Hills	K01	T01	H01
65	IT18	13	*Quercus pubescens*	Italy	Veneto	Euganei Hills	K01	T01	H01
66	IT18	14	*Quercus pubescens*	Italy	Veneto	Euganei Hills	K01	T01	H01
67	IT19	1	*Quercus dalechampii*	Italy	Veneto	Euganei Hills	K01	T01	H01
68	IT19	2	*Quercus dalechampii*	Italy	Veneto	Euganei Hills	K01	T01	H01
69	IT19	3	*Quercus dalechampii*	Italy	Veneto	Euganei Hills	K01	T01	H01
70	IT20	1	*Quercus petraea*	Italy	Veneto	Euganei Hills	K01	T01	H01
71	IT20	2	*Quercus petraea*	Italy	Veneto	Euganei Hills	K01	T01	H01
72	IT20	3	*Quercus petraea*	Italy	Veneto	Euganei Hills	K01	T01	H01
73	IT21	1	*Quercus robur*	Italy	Veneto	Euganei Hills	K01	T01	H01
74	IT21	2	*Quercus robur*	Italy	Veneto	Euganei Hills	K01	T01	H01
75	IT21	3	*Quercus robur*	Italy	Veneto	Euganei Hills	K01	T01	H01
76	IT22	1	*Quercus pubescens*	Italy	Lombardy	One, San Martino (BS)	K02	T03	H03
77	IT22	2	*Quercus pubescens*	Italy	Lombardy	One, San Martino (BS)	K02	T03	H03
78	IT22	3	*Quercus pubescens*	Italy	Lombardy	One, San Martino (BS)	K01	T01	H01
79	IT23	1	*Quercus pubescens*	Italy	Lazio	Monte San Biagio Plain	K02	T04	H04
80	IT23	2	*Quercus pubescens*	Italy	Lazio	Monte San Biagio Plain	K02	T04	H04
81	IT23	3	*Quercus pubescens*	Italy	Lazio	Monte San Biagio Plain	K02	T04	H04
82	IT24	1	*Quercus frainetto*	Italy	Lazio	Selvapiana Wood, Amaseno	K02	T01	H06
83	IT24	2	*Quercus frainetto*	Italy	Lazio	Selvapiana Wood, Amaseno	K02	T01	H06
84	IT24	3	*Quercus frainetto*	Italy	Lazio	Selvapiana Wood, Amaseno	K02	T01	H06
85	IT25	1	*Quercus virgiliana*	Italy	Sicily	Fitalia Valley (Frazzanò, ME)	K02	T03	H03
86	IT25	2	*Quercus virgiliana*	Italy	Sicily	Fitalia Valley (Frazzanò, ME)	K02	T03	H03
87	IT25	3	*Quercus virgiliana*	Italy	Sicily	Fitalia Valley (Frazzanò, ME)	K02	T03	H03
88	IT26	1	*Quercus frainetto*	Italy	Lazio	Aurunci	K02	T04	H04
89	IT26	2	*Quercus frainetto*	Italy	Lazio	Aurunci	K02	T04	H04
90	IT26	3	*Quercus frainetto*	Italy	Lazio	Aurunci	K02	T04	H04

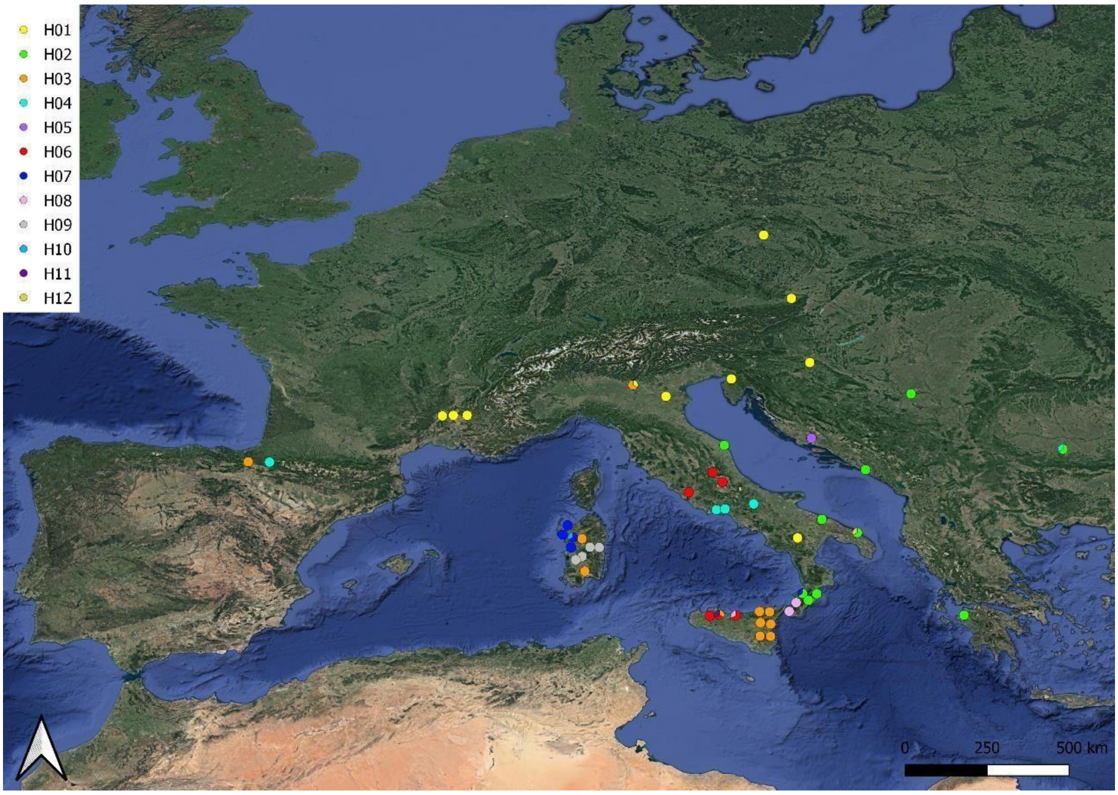
91	IT27	1	*Quercus dalechampii*	Italy	Calabria	Serre, Sant'Angelo SS182 (VV)	K01	T02	H02
92	IT27	2	*Quercus dalechampii*	Italy	Calabria	Serre, Sant'Angelo SS182 (VV)	K01	T02	H02
93	IT27	3	*Quercus dalechampii*	Italy	Calabria	Serre, Sant'Angelo SS182 (VV)	K04	T05	H07
94	IT28	1	*Quercus dalechampii*	Italy	Calabria	Aspromonte, SP Palmi Pontevecchio. Croce Mammone (Cirello), Rizziconi (RC)	K02	T06	H08
95	IT28	2	*Quercus dalechampii*	Italy	Calabria	Aspromonte, SP Palmi Pontevecchio. Croce Mammone (Cirello), Rizziconi (RC)	K02	T06	H08
96	IT28	3	*Quercus dalechampii*	Italy	Calabria	Aspromonte, SP Palmi Pontevecchio. Croce Mammone (Cirello), Rizziconi (RC)	K02	T06	H08
97	IT29	1	*Quercus dalechampii*	Italy	Calabria	Aspromonte, SP 3 towards Piani di Carmelia	K02	T06	H08
98	IT29	2	*Quercus dalechampii*	Italy	Calabria	Aspromonte, SP 3 towards Piani di Carmelia	K02	T06	H08
99	IT29	3	*Quercus dalechampii*	Italy	Calabria	Aspromonte, SP 3 towards Piani di Carmelia	K02	T06	H08
100	CZ30	1	*Quercus pubescens*	Czech Republic		Milovice	K01	T01	H01
101	CZ30	2	*Quercus pubescens*	Czech Republic		Milovice	K01	T01	H01
102	CZ30	3	*Quercus pubescens*	Czech Republic		Milovice	K01	T01	H01
103	CZ32	1	*Quercus robur*	Czech Republic		Lednice	K01	T01	H01
104	CZ32	2	*Quercus robur*	Czech Republic		Lednice	K01	T01	H01
105	CZ32	3	*Quercus robur*	Czech Republic		Lednice	K01	T01	H01
106	RS34	1	*Quercus frainetto*	Serbia		Tara Mountain	K01	T02	H02
107	RS34	2	*Quercus frainetto*	Serbia		Tara Mountain	K01	T02	H02
108	RS34	3	*Quercus frainetto*	Serbia		Tara Mountain	K01	T02	H02
109	RS35	1	*Quercus pubescens*	Serbia		Grgurevci	K01	T02	H02
110	RS35	2	*Quercus pubescens*	Serbia		Grgurevci	K01	T02	H02
111	RS35	3	*Quercus pubescens*	Serbia		Grgurevci	K01	T02	H02
112	RS36	1	*Quercus petraea*	Serbia		Vrdnik	K01	T02	H02
113	RS36	2	*Quercus petraea*	Serbia		Vrdnik	K01	T02	H02
114	RS36	3	*Quercus petraea*	Serbia		Vrdnik	K01	T02	H02
115	RS38	1	*Quercus dalechampii*	Serbia		Mt. Suvobor	K01	T02	H02
116	RS38	2	*Quercus dalechampii*	Serbia		Mt. Suvobor	K01	T02	H02
117	RS38	3	*Quercus dalechampii*	Serbia		Mt. Suvobor	K01	T02	H02
118	RS39	1	*Quercus robur*	Serbia		Mt. Suvobor	K01	T02	H02
119	RS39	2	*Quercus robur*	Serbia		Mt. Suvobor	K01	T02	H02
120	RS39	3	*Quercus robur*	Serbia		Mt. Suvobor	K01	T02	H02
121	ES40	1	*Quercus pyrenaica*	Spain		Ranera, Partido de la Sierra en Tobalina, Burgos	K02	T04	H04
122	ES40	2	*Quercus pyrenaica*	Spain		Ranera, Partido de la Sierra en Tobalina, Burgos	K02	T04	H04
123	ES40	3	*Quercus pyrenaica*	Spain		Ranera, Partido de la Sierra en Tobalina, Burgos	K02	T04	H04
124	ES41	1	*Quercus robur*	Spain		Leioa	K02	T03	H03
125	ES41	2	*Quercus robur*	Spain		Leioa	K02	T04	H04
126	ES41	3	*Quercus robur*	Spain		Leioa	K02	T04	H04
127	ES42	1	*Quercus faginea*	Spain		Zaldiaran	K02	T03	H03
128	ES42	3	*Quercus faginea*	Spain		Zaldiaran	K02	T04	H04
129	ES42	5	*Quercus faginea*	Spain		Zaldiaran	K02	T04	H04
130	ES44	1	*Quercus pubescens*	Spain		Madotz	K02	T03	H03
131	ES44	2	*Quercus pubescens*	Spain		Madotz	K02	T03	H03
132	ES44	3	*Quercus pubescens*	Spain		Madotz	K02	T03	H03
133	ES45	1	*Quercus pubescens*	Spain		Zilbeti	K02	T04	H04
134	ES45	2	*Quercus pubescens*	Spain		Zilbeti	K02	T04	H04
135	ES45	3	*Quercus pubescens*	Spain		Zilbeti	K02	T04	H04
136	IT46	1	*Quercus petraea*	Italy	Lazio	Mt. Cimini	K01	T02	H02
137	IT46	2	*Quercus petraea*	Italy	Lazio	Mt. Cimini	K01	T02	H02
138	IT46	3	*Quercus petraea*	Italy	Lazio	Mt. Cimini	K01	T02	H02
139	IT48	1	*Quercus ichnusae*	Italy	Sardinia	Senis	K02	T07	H09
140	IT48	2	*Quercus ichnusae*	Italy	Sardinia	Senis	K02	T07	H09
141	IT48	3	*Quercus ichnusae*	Italy	Sardinia	Senis	K02	T07	H09
142	IT49	34	*Quercus virgiliana*	Italy	Sardinia	Pau	K02	T07	H09
143	IT49	35	*Quercus virgiliana*	Italy	Sardinia	Pau	K02	T07	H09
144	IT49	36	*Quercus virgiliana*	Italy	Sardinia	Pau	K02	T07	H09
145	IT50	71	*Quercus ichnusae*	Italy	Sardinia	Monte Traessu	K04	T05	H07
146	IT50	72	*Quercus ichnusae*	Italy	Sardinia	Monte Traessu	K02	T05	H10
147	IT50	73	*Quercus ichnusae*	Italy	Sardinia	Monte Traessu	K04	T05	H07
148	IT51	94	*Quercus virgiliana*	Italy	Sardinia	Ittiri	K04	T05	H07
149	IT51	95	*Quercus virgiliana*	Italy	Sardinia	Ittiri	K04	T05	H07
150	IT51	96	*Quercus virgiliana*	Italy	Sardinia	Ittiri	K04	T05	H07
151	IT52	118	*Quercus congesta*	Italy	Sardinia	Fonni (Muggiana)	K02	T07	H09
152	IT52	119	*Quercus congesta*	Italy	Sardinia	Fonni (Muggiana)	K02	T07	H09
153	IT52	120	*Quercus congesta*	Italy	Sardinia	Fonni (Muggiana)	K02	T07	H09
154	IT53	140	*Quercus congesta*	Italy	Sardinia	Fonni (Govossai)	K02	T07	H09
155	IT53	141	*Quercus congesta*	Italy	Sardinia	Fonni (Govossai)	K02	T07	H09
156	IT53	142	*Quercus congesta*	Italy	Sardinia	Fonni (Govossai)	K02	T07	H09
157	IT54	1	*Quercus leptobalanos*	Italy	Sicily	Ficuzza Wood (PA)	K02	T01	H06
158	IT54	2	*Quercus leptobalanos*	Italy	Sicily	Ficuzza Wood (PA)	K02	T01	H06
159	IT54	3	*Quercus leptobalanos*	Italy	Sicily	Ficuzza Wood (PA)	K02	T01	H06
160	IT55	1	*Quercus virgiliana*	Italy	Sicily	Marineo (PA)	K05	T01	H11
161	IT55	2	*Quercus virgiliana*	Italy	Sicily	Marineo (PA)	K02	T01	H06
162	IT55	3	*Quercus virgiliana*	Italy	Sicily	Marineo (PA)	K02	T03	H03
163	IT56	1	*Quercus congesta*	Italy	Sicily	Etna	K02	T03	H03
164	IT56	2	*Quercus congesta*	Italy	Sicily	Etna	K02	T03	H03
165	IT56	3	*Quercus congesta*	Italy	Sicily	Etna	K02	T03	H03
166	IT57	1	*Quercus dalechampii*	Italy	Sicily	Etna	K02	T03	H03
167	IT57	2	*Quercus dalechampii*	Italy	Sicily	Etna	K02	T03	H03
168	IT57	3	*Quercus dalechampii*	Italy	Sicily	Etna	K02	T03	H03
169	IT58	1	*Quercus virgiliana*	Italy	Sicily	Etna. Mt. Ceraulo, Mascalucia (CT)	K02	T03	H03
170	IT58	2	*Quercus virgiliana*	Italy	Sicily	Etna. Mt. Ceraulo, Mascalucia (CT)	K02	T03	H03
171	IT58	3	*Quercus virgiliana*	Italy	Sicily	Etna. Mt. Ceraulo, Mascalucia (CT)	K02	T03	H03
172	IT59	1	*Quercus virgiliana*	Italy	Sicily	Etna. Tre castagni, Via P. Togliatti (CT)	K02	T03	H03
173	IT59	2	*Quercus virgiliana*	Italy	Sicily	Etna. Tre castagni, Via P. Togliatti (CT)	K02	T03	H03
174	IT59	3	*Quercus virgiliana*	Italy	Sicily	Etna. Tre castagni, Via P. Togliatti (CT)	K02	T03	H03
175	IT60	1	*Quercus congesta*	Italy	Sicily	Madonie, between Piano Torre and Piano Zucchi (Collesano, PA)	K02	T06	H08
176	IT60	2	*Quercus congesta*	Italy	Sicily	Madonie, between Piano Torre and Piano Zucchi (Collesano, PA)	K02	T01	H06
177	IT60	3	*Quercus congesta*	Italy	Sicily	Madonie, between Piano Torre and Piano Zucchi (Collesano, PA)	K02	T01	H06
178	IT61	1	*Quercus congesta*	Italy	Sicily	Valley of Flascio, Nebrodi	K02	T03	H03
179	IT61	2	*Quercus congesta*	Italy	Sicily	Valley of Flascio, Nebrodi	K02	T03	H03
180	IT61	3	*Quercus congesta*	Italy	Sicily	Valley of Flascio, Nebrodi	K02	T03	H03

181	IT62	1	*Quercus robur*	Italy	Apulia	Lake of Laterza, Tajuri (BA)	K01	T01	H01
182	IT62	2	*Quercus robur*	Italy	Apulia	Lake of Laterza, Tajuri (BA)	K01	T01	H01
183	IT62	3	*Quercus robur*	Italy	Apulia	Lake of Laterza, Tajuri (BA)	K01	T01	H01
184	IT63	1	*Quercus dalechampii*	Italy	Calabria	Serre, Sant'Angelo SS182 crossroads SP Nardodipace (VV)	K01	T02	H02
185	IT63	2	*Quercus dalechampii*	Italy	Calabria	Serre, Sant'Angelo SS182 crossroads SP Nardodipace (VV)	K01	T02	H02
186	IT63	3	*Quercus dalechampii*	Italy	Calabria	Serre, Sant'Angelo SS182 crossroads SP Nardodipace (VV)	K01	T02	H02
187	IT64	1	*Quercus pubescens*	Italy	Lazio	Aurunci	K02	T04	H04
188	IT64	2	*Quercus pubescens*	Italy	Lazio	Aurunci	K02	T04	H04
189	IT64	3	*Quercus pubescens*	Italy	Lazio	Aurunci	K02	T04	H04
190	AT01	30	*Quercus pubescens*	Austria		Leopoldsberg, Vienna	K01	T01	H01
191	AT01	31	*Quercus pubescens*	Austria		Leopoldsberg, Vienna	K01	T01	H01
192	AT01	33	*Quercus pubescens*	Austria		Leopoldsberg, Vienna	K01	T01	H01
193	IT65	1	*Quercus robur*	Italy	Apulia	Lake of Laterza, Tajuri (BA)	K01	T01	H01
194	IT65	2	*Quercus robur*	Italy	Apulia	Lake of Laterza, Tajuri (BA)	K01	T01	H01
195	IT65	3	*Quercus robur*	Italy	Apulia	Lake of Laterza, Tajuri (BA)	K01	T01	H01
196	IT66	4	*Quercus virgiliana*	Italy	Apulia	Lake of Laterza, Tajuri (BA)	K01	T02	H02
197	IT66	5	*Quercus virgiliana*	Italy	Apulia	Lake of Laterza, Tajuri (BA)	K01	T02	H02
198	IT66	6	*Quercus virgiliana*	Italy	Apulia	Lake of Laterza, Tajuri (BA)	K01	T02	H02
199	RS67	1	*Quercus dalechampii*	Serbia		Kopaonik National Park	K01	T02	H02
200	RS67	12	*Quercus dalechampii*	Serbia		Kopaonik National Park	K01	T02	H02
201	RS67	14	*Quercus dalechampii*	Serbia		Kopaonik National Park	K01	T02	H02
202	IT68	1	*Quercus pubescens*	Italy	Sardinia	Mt. Zara, Monastir (CA)	K02	T03	H03
203	IT68	2	*Quercus pubescens*	Italy	Sardinia	Mt. Zara, Monastir (CA)	K02	T03	H03
204	IT68	3	*Quercus pubescens*	Italy	Sardinia	Mt. Zara, Monastir (CA)	K02	T03	H03
205	IT69	11	*Quercus ichnusae*	Italy	Sardinia	S. Antonio Wood, Macomer (NU)	K04	T05	H07
206	IT69	12	*Quercus ichnusae*	Italy	Sardinia	S. Antonio Wood, Macomer (NU)	K04	T05	H07
207	IT69	13	*Quercus ichnusae*	Italy	Sardinia	S. Antonio Wood, Macomer (NU)	K04	T05	H07
208	IT70	26	*Quercus congesta*	Italy	Sardinia	Sant'Orsola (SS)	K04	T05	H07
209	IT70	27	*Quercus congesta*	Italy	Sardinia	Sant'Orsola (SS)	K04	T05	H07
210	IT70	28	*Quercus congesta*	Italy	Sardinia	Sant'Orsola (SS)	K04	T05	H07
211	IT71	31	*Quercus congesta*	Italy	Sardinia	Mt. Rasu, Catena del Marghine, Bono (SS)	K02	T03	H03
212	IT71	32	*Quercus congesta*	Italy	Sardinia	Mt. Rasu, Catena del Marghine, Bono (SS)	K02	T03	H03
213	IT71	33	*Quercus congesta*	Italy	Sardinia	Mt. Rasu, Catena del Marghine, Bono (SS)	K02	T03	H03
214	IT72	1	*Quercus pubescens*	Italy	Basilicata	Laurenzana (PZ)	K01	T01	H01
215	IT72	2	*Quercus pubescens*	Italy	Basilicata	Laurenzana (PZ)	K01	T01	H01
216	IT72	3	*Quercus pubescens*	Italy	Basilicata	Laurenzana (PZ)	K01	T01	H01
217	IT73	1	*Quercus petraea*	Italy	Molise	Monte Vairano	K02	T04	H04
218	IT73	4	*Quercus petraea*	Italy	Molise	Monte Vairano	K01	T02	H02
219	IT73	5	*Quercus petraea*	Italy	Molise	Monte Vairano	K01	T02	H02
220	IT75	1	*Quercus pubescens*	Italy	Molise	Monte Vairano	K02	T04	H04
221	IT75	2	*Quercus pubescens*	Italy	Molise	Monte Vairano	K02	T04	H04
222	IT75	3	*Quercus pubescens*	Italy	Molise	Monte Vairano	K02	T04	H04
223	IT76	1	*Quercus pubescens*	Italy	Calabria	Nardodipace	K01	T02	H02
224	IT76	2	*Quercus pubescens*	Italy	Calabria	Nardodipace	K01	T02	H02
225	IT76	3	*Quercus pubescens*	Italy	Calabria	Nardodipace	K01	T02	H02
226	IT77	5	*Quercus pubescens*	Italy	Apulia	Bosco del Compare	K01	T02	H02
227	IT77	7	*Quercus pubescens*	Italy	Apulia	Bosco del Compare	K01	T02	H02
228	IT77	12	*Quercus pubescens*	Italy	Apulia	Bosco del Compare	K01	T04	H12
229	IT82	5	*Quercus pubescens*	Italy	Lazio	Torrita, Laga Mountains (Amatrice, RI)	K02	T01	H06
230	IT82	6	*Quercus pubescens*	Italy	Lazio	Torrita, Laga Mountains (Amatrice, RI)	K02	T01	H06
231	IT82	7	*Quercus pubescens*	Italy	Lazio	Torrita, Laga Mountains (Amatrice, RI)	K02	T01	H06
232	IT83	1	*Quercus pubescens*	Italy	Abruzzo	Barisciano, San Colombo (AQ)	K02	T01	H06
233	IT83	3	*Quercus pubescens*	Italy	Abruzzo	Barisciano, San Colombo (AQ)	K02	T01	H06
234	IT83	4	*Quercus pubescens*	Italy	Abruzzo	Barisciano, San Colombo (AQ)	K02	T01	H06
235	ES85	1	*Quercus faginea*	Spain		Cordoba	K02	T04	H04
236	ES85	2	*Quercus faginea*	Spain		Cordoba	K02	T08	H13
237	ES85	3	*Quercus faginea*	Spain		Cordoba	K02	T09	H14
238	MA86	1	*Quercus faginea*	Morocco		Ahfir	K02	T04	H04
239	MA86	2	*Quercus faginea*	Morocco		Ahfir	K02	T04	H04
240	MA86	3	*Quercus faginea*	Morocco		Ahfir	K02	T04	H04
241	IT84	1	*Quercus robur*	Italy	Lombardy	Groane Park, Solaro (MI)	K02	T03	H03
242	IT84	2	*Quercus robur*	Italy	Lombardy	Groane Park, Solaro (MI)	K02	T03	H03
243	IT84	3	*Quercus robur*	Italy	Lombardy	Groane Park, Solaro (MI)	K02	T01	H06
244	IT85	1	*Quercus robur*	Italy	Lombardy	Bosco Fontana, Marmirolo (MN)	K01	T01	H01
245	IT85	4	*Quercus robur*	Italy	Lombardy	Bosco Fontana, Marmirolo (MN)	K02	T03	H03
246	IT85	5	*Quercus robur*	Italy	Lombardy	Bosco Fontana, Marmirolo (MN)	K01	T01	H01
247	IT86	1	*Quercus robur*	Italy	Piedmont	Natural Park La Mandria, Venaria Reale (TO)	K01	T01	H01
248	IT86	2	*Quercus robur*	Italy	Piedmont	Natural Park La Mandria, Venaria Reale (TO)	K01	T01	H01
249	IT86	4	*Quercus robur*	Italy	Piedmont	Natural Park La Mandria, Venaria Reale (TO)	K01	T01	H01
250	IT94	1	*Quercus petraea*	Italy	Umbria	Montecorona, Umbertide (PG)	K02	T04	H04
251	IT94	2	*Quercus petraea*	Italy	Umbria	Montecorona, Umbertide (PG)	K02	T04	H04
252	IT94	3	*Quercus petraea*	Italy	Umbria	Montecorona, Umbertide (PG)	K02	T04	H04
253	IT95	1	*Quercus petraea*	Italy	Tuscany	Montefalcone Natural Reserve, Castelfranco di Sotto (PI)	K01	T01	H01
254	IT95	2	*Quercus petraea*	Italy	Tuscany	Montefalcone Natural Reserve, Castelfranco di Sotto (PI)	K01	T01	H01
255	IT95	3	*Quercus petraea*	Italy	Tuscany	Montefalcone Natural Reserve, Castelfranco di Sotto (PI)	K01	T01	H01
256	IT96	2	*Quercus petraea*	Italy	Emilia-Romagna	Parma	K02	T03	H03
257	IT96	3	*Quercus petraea*	Italy	Emilia-Romagna	Parma	K02	T03	H03
258	IT96	4	*Quercus petraea*	Italy	Emilia-Romagna	Parma	K02	T03	H03
259	IT90	1	*Quercus frainetto*	Italy	Umbria	S.I.C. IT5210077, Collestrada (PG)	K01	T02	H02
260	IT90	2	*Quercus frainetto*	Italy	Umbria	S.I.C. IT5210077, Collestrada (PG)	K01	T02	H02
261	IT90	3	*Quercus frainetto*	Italy	Umbria	S.I.C. IT5210077, Collestrada (PG)	K01	T02	H02
262	IT91	1	*Quercus frainetto*	Italy	Calabria	National Park Aspromonte, Platì (RC)	K01	T02	H02
263	IT91	2	*Quercus frainetto*	Italy	Calabria	National Park Aspromonte, Platì (RC)	K01	T02	H02
264	IT91	3	*Quercus frainetto*	Italy	Calabria	National Park Aspromonte, Platì (RC)	K01	T02	H02
265	IT92	1	*Quercus pubescens*	Italy	Friuli V. Giulia	Basovizza (TS)	K01	T01	H01
266	IT92	2	*Quercus pubescens*	Italy	Friuli V. Giulia	Basovizza (TS)	K01	T01	H01
267	IT92	3	*Quercus pubescens*	Italy	Friuli V. Giulia	Basovizza (TS)	K01	T01	H01
268	IT93	1	*Quercus pubescens*	Italy	Marche	Selva of Castelfidardo (AN)	K01	T02	H02
269	IT93	2	*Quercus pubescens*	Italy	Marche	Selva of Castelfidardo (AN)	K01	T02	H02
270	IT93	3	*Quercus pubescens*	Italy	Marche	Selva of Castelfidardo (AN)	K01	T02	H02

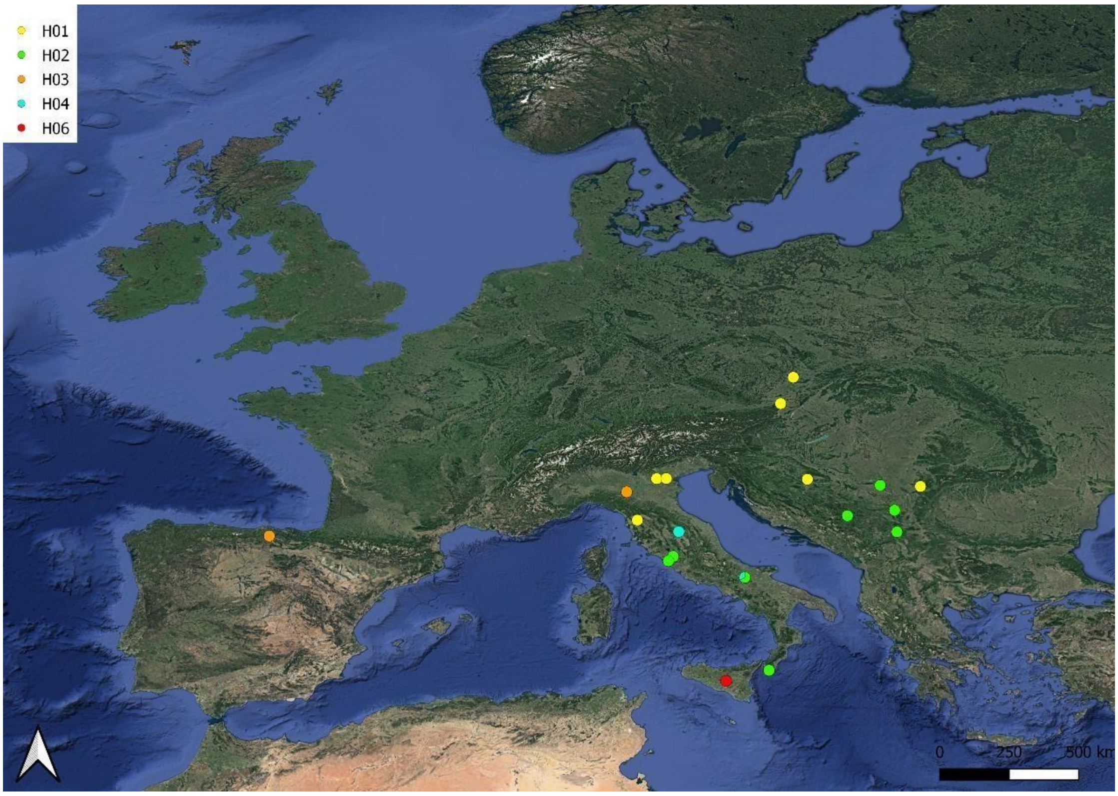
Supplementary File 6a Geographic distribution of the plastid haplotypes in the populations of *Quercus* sampled across Italy



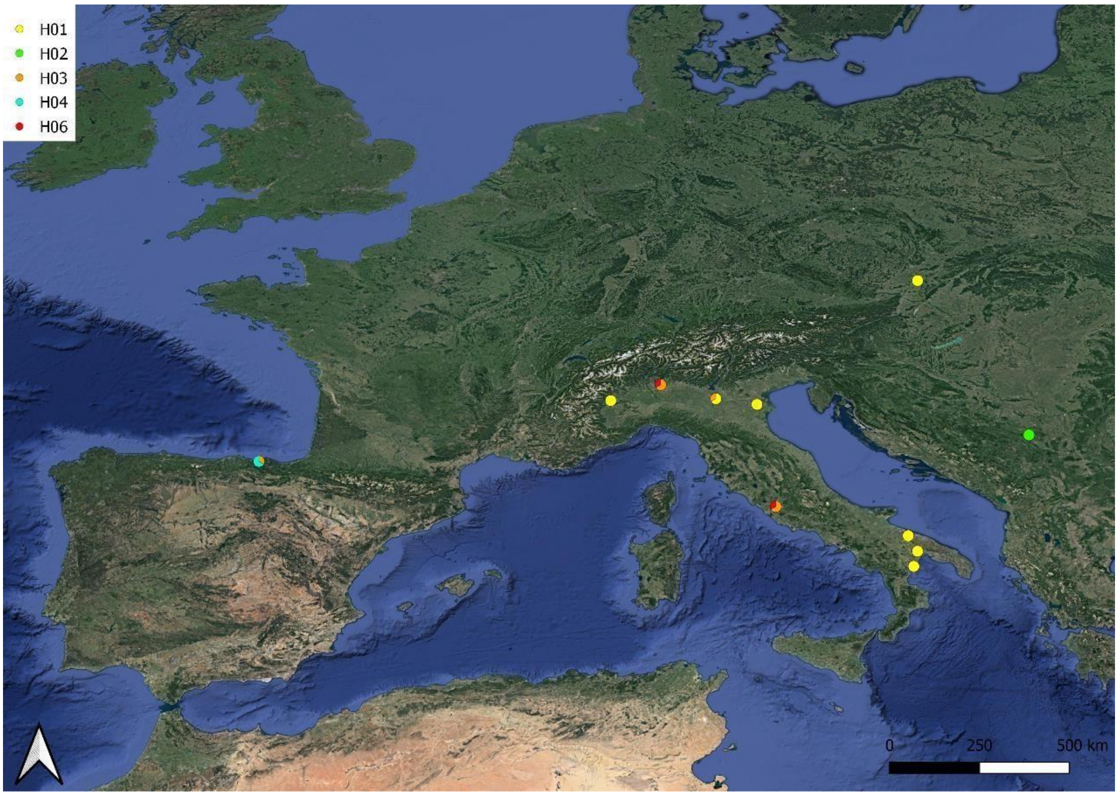
Supplementary File 6b Geographic distribution of the plastid haplotypes in the populations of *Quercus pubescens s.l.* (*Q. congesta*, *Q. dalechampii* (*Q. pubescens* group), *Q. ichnusae*, *Q. leptobalanos*, *Q. pubescens s.s.*, *Q. virgiliana*) sampled



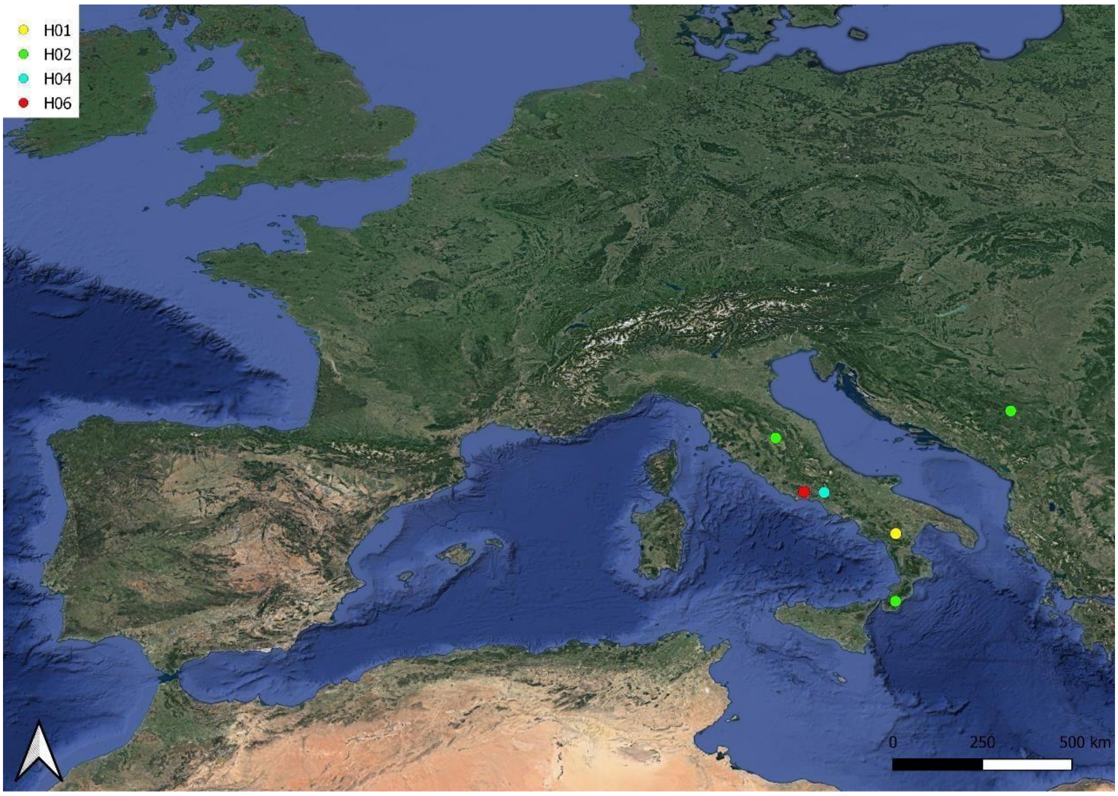
Supplementary File 6c Geographic distribution of the plastid haplotypes in the populations of *Quercus petraea s.l.* (*Q. dalechampii* (*Q. petraea* group), *Q. petraea s.s.*, *Q. petraea* subsp. *austrotyrrhenica*) sampled



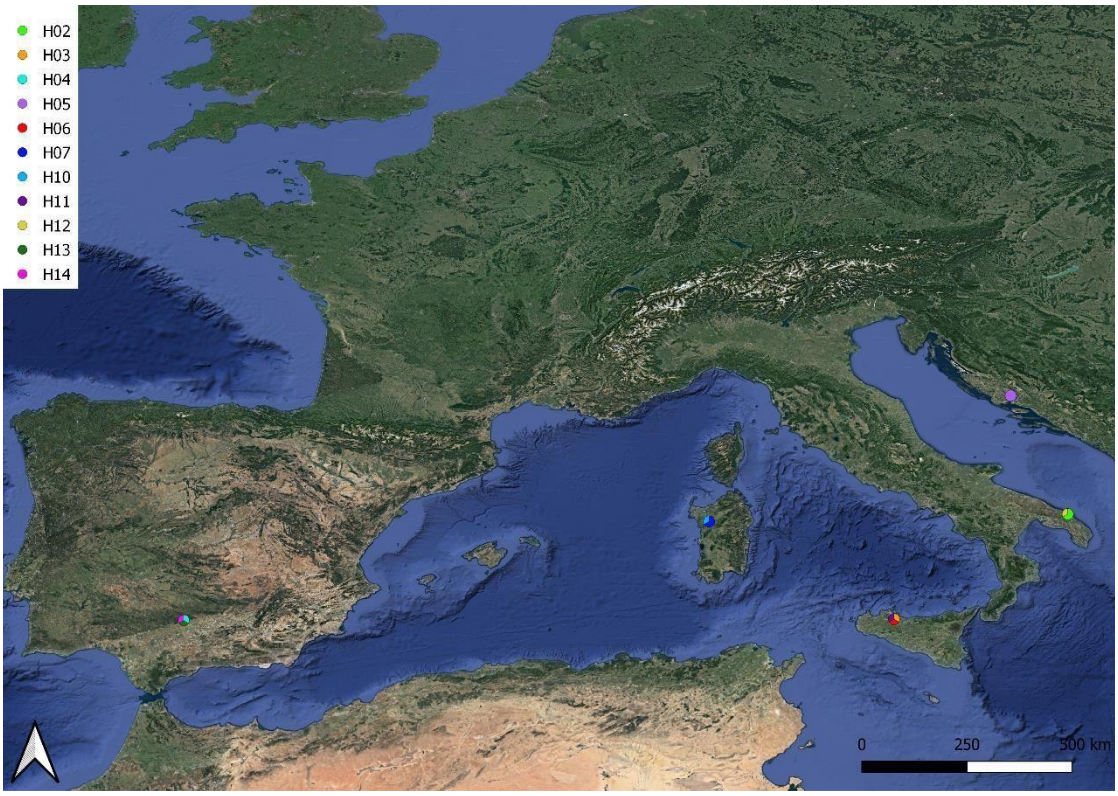
Supplementary File 6d Geographic distribution of the plastid haplotypes in the populations of *Quercus robur* sampled



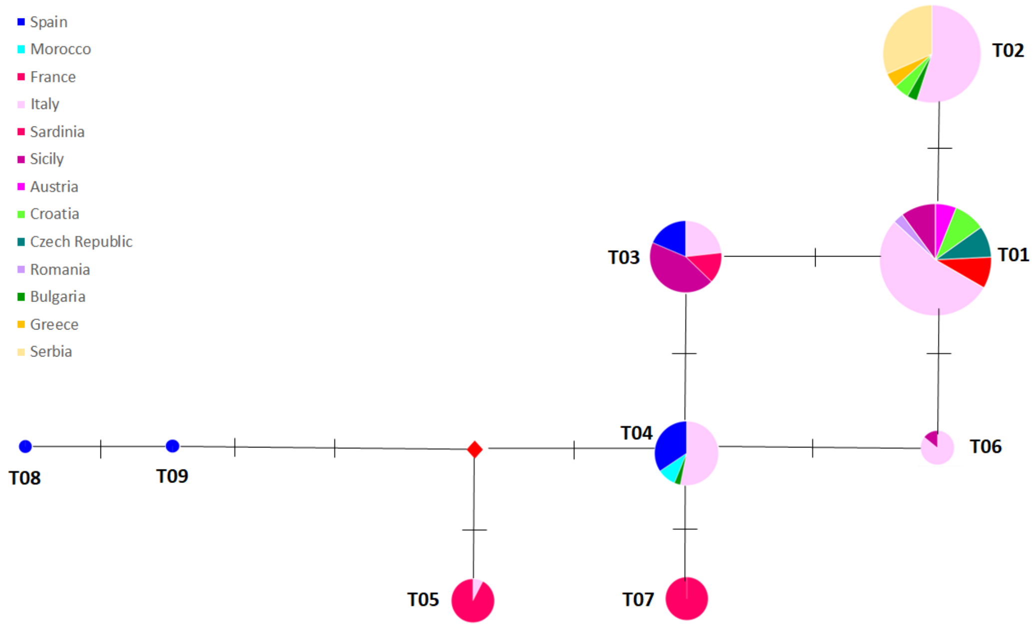
Supplementary File 6e Geographic distribution of the plastid haplotypes in the populations of *Quercus frainetto* sampled



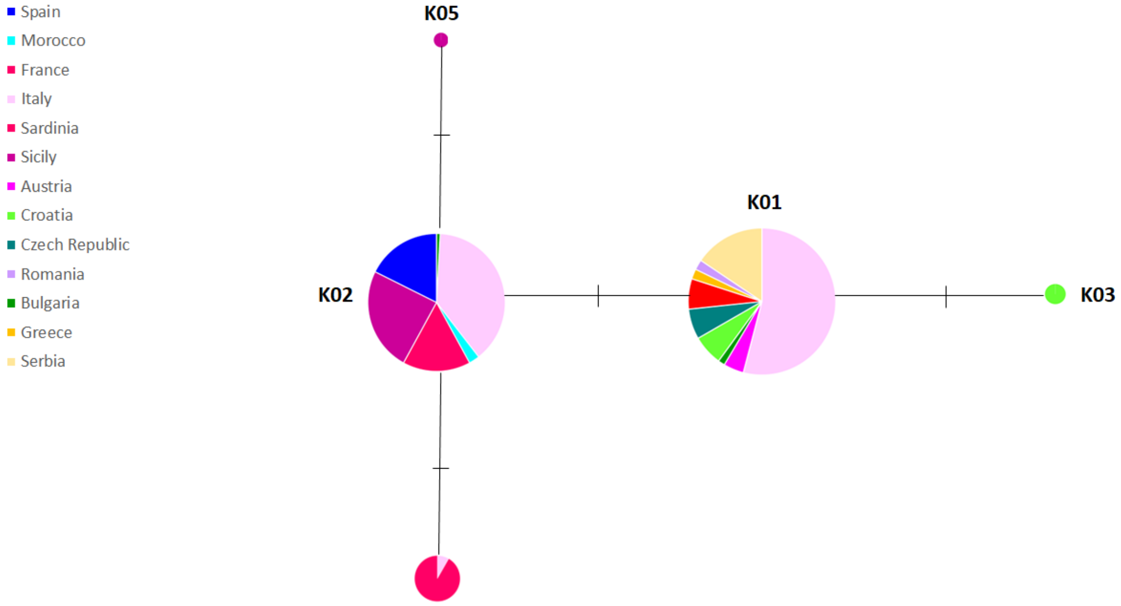
Supplementary File 6f Geographic distribution of the rare plastid haplotypes (H05, H10, H11, H12, H13, H14) in the populations of *Quercus* sampled



Supplementary File 7a Median-joining network based on the plastid locus *trn*H-*psb*A of the 270 samples of the *Quercus* genus analysed. Each sequenced haplotype is represented by a circle, the size of which is proportional to its overall frequency in the dataset (Supplementary File 5). The number of mutational changes between two distinct haplotypes is shown by the lines on the branches. The red diamond represents the median vector. Haplotype colours correspond to the sampling localities



Supplementary File 7b Median-joining network based on the plastid locus *trn*K-*mat*K of the 270 samples of the *Quercus* genus analysed. Each sequenced haplotype is represented by a circle, the size of which is proportional to its overall frequency in the dataset (Supplementary File 5). The number of mutational changes between two distinct haplotypes is shown by the lines on the branches. Haplotype colours correspond to the sampling localities



Supplementary File 8 Frequency of null alleles per population and SSR locus calculated in FREENA

No. Population	**ID Population**	**Taxon**	**PIE227**	**PIE223**	**PIE215**	**PIE20**	**PIE152**	**PIE243**	**PIE242**	**PIE267**	**PIE102**	**PIE271**
1	**IT18**	*Quercus pubescens*	0	0.0869	0	0	0.0766	0	0.0017	0.0003	0.1041	0.0607
2	**IT22**	*Quercus pubescens*	0.0149	0	0	0	2E-05	3E-05	0.0582	0.0103	3E-05	0.1079
3	**IT15**	*Quercus pubescens*	4E-05	0	0.0014	0.0909	0	1E-05	0.1106	0.0619	0	0
4	**IT23**	*Quercus pubescens*	1E-05	0.0004	0	5E-05	0.0496	0.0637	0	0.0649	1E-05	1E-05
5	**IT64**	*Quercus pubescens*	0	0.052	0.0289	0	0	0.0524	0.1013	0.1128	0	0.0514
8	**IT87**	*Quercus pubescens*	0.0413	0.1568	0	0.0283	0.0722	0	0.0132	0.0344	0	0.064
9	**IT88**	*Quercus pubescens*	0.0649	0.0562	0.0418	0.0388	0.2094	0.1181	0	0.1	0.1147	0.0278
10	**IT89**	*Quercus pubescens*	0	0.0661	0.074	0	0.145	0.0343	0	0	0.0842	0.0854
12	**IT48**	*Quercus ichnusae*	2E-05	0.0505	0.0561	0	0	0	0.0347	0	0.0461	0
13	**IT49**	*Quercus virgiliana*	0.001	0.134	0.0309	0	0.058	0.0495	0.028	0	0.0782	1E-05
14	**IT50**	*Quercus ichnusae*	1E-05	0	0.0085	0	0.0049	0.0209	1E-05	0	0.0512	0
15	**IT51**	*Quercus virgiliana*	0	0.0641	0.0124	0	1E-05	0.0482	0.0928	0.0307	0.0221	2E-05
16	**IT52**	*Quercus congesta*	2E-05	0.141	0.0084	0.0033	0.018	1E-05	1E-05	0.0509	0	0.018
17	**IT53**	*Quercus congesta*	1E-05	0	0	0	0	0.0194	0	0	1E-05	7E-05
18	**ES44**	*Quercus pubescens*	0.0102	0	0.0898	0.1061	0.0212	1E-05	0.2298	0.0645	0.0195	0.0836
19	**ES45**	*Quercus pubescens*	0.0838	0	0.0281	0	0	0.0258	0	0	0.014	0
20	**FR08**	*Quercus pubescens*	0	0.0257	1E-05	0	0	0	0.0097	0	0	0
21	**FR09**	*Quercus pubescens*	0.0636	1E-05	0.0752	0	0.1068	1E-05	0	0.007	0	0.0692
22	**FR10**	*Quercus pubescens*	0	0.038	0.0198	0	1E-05	1E-05	0.0543	1E-05	0.0003	0
23	**AT01**	*Quercus pubescens*	1E-05	0.0289	3E-05	0	0	0.0092	0	0.0456	0.1035	0
25	**HR04**	*Quercus pubescens*	0	0.0529	0.0499	0	0	0.0964	0.0061	0.0473	2E-05	0
26	**HR05**	*Quercus pubescens*	0.0673	0	0.0223	0	0	0	0	0	0	9E-05
27	**HR07**	*Quercus pubescens*	0.0844	0	0.0343	0	0.0886	0	0	0	0.1073	0.0878
28	**GR11**	*Quercus pubescens*	0.0491	0.0511	0.0123	0.1221	1E-05	0.0167	0	0.1386	0.0931	0.096
29	**CZ30**	*Quercus pubescens*	0	0.0158	8E-05	0	0.0245	1E-05	0	0	0	0.0266
30	**RS35**	*Quercus pubescens*	0	0	0	0	0.1	0	0.0212	0	0	1E-05
31	**IT19**	*Quercus dalechampii*	0.0372	0.0593	0.1204	0	0.0386	4E-05	0	0.1068	0	0.0026
32	**IT20**	*Quercus petraea*	0	0.0593	0.0813	4E-05	0	0	1E-05	0.01	0	0.04
33	**IT46**	*Quercus petraea*	0	0	0.0101	0.0038	0.0858	0.0185	0	0.0503	0.0005	0
34	**IT47**	*Quercus petraea*	0	0	1E-05	0	0.0597	0.0323	0.0475	0	0	0.0271
35	**IT17**	*Quercus petraea subsp . austrotyrrhenica*	1E-05	0	0	0	0.0802	1E-05	0.0139	0	8E-05	0.0325
36	**IT16**	*Quercus petraea subsp . austrotyrrhenica*	0	0.032	0.073	0	0.1997	0	0.0282	0	0.0864	0.1058
37	**ES43**	*Quercus petraea*	2E-05	0.0831	1E-05	6E-05	0.1069	0.0159	0.0835	0.0253	0	0
38	**AT02**	*Quercus petraea*	0.0937	0	0	0	0	0	0.0665	0	0	0
39	**HR06**	*Quercus petraea*	0	0	0	0	0.0281	0.0038	0.0251	0	0.0155	0.1105
40	**CZ31**	*Quercus petraea*	0	0	1E-05	0.0155	1E-05	0	0.041	0	0	0
41	**RO33**	*Quercus banatus*	0	0.0202	0	0.0029	0.0047	0	0	0.0217	0	0.0227
42	**RS36**	*Quercus petraea*	0	0	1E-05	0	3E-05	0	0.0586	0.0611	0	3E-05
43	**RS37**	*Quercus dalechampii*	0.0414	1E-05	0.0167	0.044	0.0066	0.0063	1E-05	0	1E-05	0.1305
44	**RS38**	*Quercus dalechampii*	0	0	0.0425	0.051	0	0	1E-05	0.0209	0	0
45	**RS67**	*Quercus dalechampii*	0	0.0005	0.0005	0.2019	2E-05	0	0	0	0	0
46	**ES41**	*Quercus robur*	0	0.0795	0.1049	0.1312	0.1745	0.1272	0.0392	0.1678	0.1006	0.1799
48	**IT14**	*Quercus robur*	0.0025	0.025	0.0242	0	0	0.0309	0.0388	0.0065	0.0113	0
49	**IT13**	*Quercus robur*	2E-05	1E-05	0.1368	0.0676	0.0174	0	1E-05	0.0831	0.0169	0.0964
51	**CZ32**	*Quercus robur*	0.0419	0	0	0.0207	2E-05	0	0.0132	3E-05	0	0.0307
53	**IT24**	*Quercus frainetto*	2E-05	0.0153	0.0057	0.0349	0	0.0648	0.0521	0.1113	0	0.0682
54	**IT26**	*Quercus frainetto*	0.0627	0	1E-05	0.0291	0	0.024	0	0.0752	0	1E-05
55	**IT12**	*Quercus frainetto*	1E-05	0.0138	0	0.0667	0	0	0	0.0584	0	0
56	**RS34**	*Quercus frainetto*	0.1672	0.0586	0.0273	0	0.0649	0.0213	0	0.06	1E-05	0
57	**ES40**	*Quercus pyrenaica*	0	0.1031	1E-05	0.0004	0.0607	0	0	0	0	0
58	**ES42**	*Quercus faginea*	1E-05	0.0733	0.0389	0.1768	0.0343	0.1141	0.0242	4E-05	0.1279	0.0919

Supplementary File 9 Genetic diversity indices in the five clusters identified from the genetic structure analysis, considering only populations with proportion of membership (q_i) values ≥ 0.60. **a**: Cluster 1; **b**: Cluster 2; **c**: Cluster 3; **d**: Cluster 4; **e**: Cluster 5. N_p: private alleles; N_a: mean number of alleles; A_r: allelic richness (rarefacted for 28 gene copies); H_o: observed heterozygosity; H_e: expected heterozygosity; F_{IS}: fixation coefficient

a

No. Population	**ID Population**	**No. Samples**	**Missing genotypes (%)**	N_p	N_a	A_r28	H_o	H_e	F_{IS}	**p-value (F_{IS})**	
1	**IT18**	20	0.3 (1.5%)	0	7.3	6.669	0.686	0.715	0.041	0.272	
2	**IT22**	20	0.1 (0.5%)	0	7.7	6.993	0.673	0.739	0.091	0.007	**
3	**IT15**	20	0.6 (3.0%)	0	7.8	7.061	0.651	0.704	0.077	0.041	*
4	**IT23**	20	0.2 (1.0%)	0	8.0	7.078	0.659	0.707	0.069	0.077	
20	**FR08**	20	0.6 (3.0%)	0	7.2	6.761	0.784	0.795	0.013	0.713	
21	**FR09**	20	1.0 (5.0%)	1	8.3	7.654	0.747	0.801	0.069	0.027	*
23	**AT01**	20	0.4 (2.0%)	2	7.9	7.041	0.696	0.738	0.058	0.154	
26	**HR05**	20	0.1 (0.5%)	0	7.3	6.635	0.799	0.742	-0.080	0.027	*
27	**HR07**	20	1.3 (6.5%)	0	7.5	6.949	0.695	0.735	0.056	0.095	
29	**CZ30**	20	0.0 (0.0%)	0	8.3	7.605	0.745	0.760	0.020	0.483	
30	**RS35**	20	0.1 (0.5%)	1	7.9	7.229	0.779	0.763	-0.023	0.550	
36	**IT16**	20	0.3 (1.5%)	0	6.4	6.041	0.669	0.765	0.128	0.000	***
mean values		20		0.3	7.6	6.976	0.715	0.747	0.043		

b

No. Population	**ID Population**	**No. Samples**	**Missing genotypes (%)**	N_p	N_a	A_r28	H_o	H_e	F_{IS}	**p-value (F_{IS})**	
5	**IT64**	20	0.8 (4.0%)	0	7.5	6.784	0.620	0.698	0.114	0.003	**
8	**IT87**	20	1.6 (8.0%)	0	7.5	7.045	0.668	0.734	0.093	0.013	*
9	**IT88**	20	1.4 (7.0%)	0	6.7	6.251	0.576	0.689	0.168	0.000	***
10	**IT89**	20	0.6 (3.0%)	0	6.5	6.069	0.664	0.719	0.078	0.021	*
12	**IT48**	20	0.1 (0.5%)	1	7.8	7.098	0.724	0.731	0.010	0.669	
13	**IT49**	19	0.4 (2.1%)	0	6.5	6.099	0.588	0.658	0.108	0.007	**
14	**IT50**	20	0.1 (0.5%)	0	8.0	7.329	0.709	0.745	0.049	0.141	
15	**IT51**	20	0.7 (3.5%)	0	6.9	6.395	0.694	0.738	0.062	0.097	
16	**IT52**	20	0.9 (4.5%)	0	7.9	7.208	0.668	0.740	0.099	0.005	**
17	**IT53**	20	0.5 (2.5%)	1	7.5	6.889	0.752	0.742	-0.013	0.675	
19	**ES45**	20	0.6 (3.0%)	0	7.6	7.118	0.781	0.805	0.030	0.359	
mean values		19.9		0.2	7.3	6.753	0.677	0.727	0.073		

c

No. Population	**ID Population**	**No. Samples**	**Missing genotypes (%)**	N_p	N_a	A_r28	H_o	H_e	F_{IS}	**p-value (F_{IS})**	
31	**IT19**	20	0.1 (0.5%)	1	7.3	6.536	0.658	0.716	0.083	0.031	*
32	**IT20**	20	0.0 (0.0%)	0	6.5	5.882	0.675	0.704	0.041	0.294	
33	**IT46**	20	0.3 (1.5%)	0	6.5	6.034	0.690	0.721	0.044	0.261	
35	**IT17**	20	0.5 (2.5%)	0	6.2	5.782	0.681	0.714	0.048	0.200	
37	**ES43**	20	0.3 (1.5%)	1	6.3	5.832	0.625	0.704	0.115	0.007	**
38	**AT02**	20	0.0 (0.0%)	0	7.6	6.719	0.745	0.719	-0.037	0.326	
39	**HR06**	20	0.5 (2.5%)	0	7.7	7.031	0.760	0.767	0.009	0.780	
40	**CZ31**	20	0.2 (1.0%)	0	7.4	6.783	0.786	0.756	-0.041	0.291	
41	**RO33**	20	0.0 (0.0%)	0	7.5	6.830	0.800	0.781	-0.024	0.518	
42	**RS36**	20	0.0 (0.0%)	0	7.8	7.002	0.765	0.767	0.002	0.979	
44	**RS38**	20	0.0 (0.0%)	1	6.7	6.201	0.745	0.739	-0.009	0.885	
45	**RS67**	14	0.0 (0.0%)	1	5.9	5.900	0.750	0.731	-0.027	0.564	
mean values		19.5		0.3	7.0	6.378	0.723	0.735	0.017		

d

No. Population	ID Population	No. Samples	Missing genotypes (%)	N_p	N_a	A_r28	H_o	H_e	F_{IS}	p-value (F_{IS})	
46	**ES41**	20	3.1 (15.5%)	0	6.4	6.185	0.523	0.718	0.279	0.000	***
48	**IT14**	20	0.0 (0.0%)	0	7.1	6.516	0.705	0.723	0.025	0.448	
49	**IT13**	20	0.2 (1.0%)	2	5.5	5.243	0.623	0.689	0.098	0.025	*
51	**CZ32**	20	0.1 (0.5%)	0	6.8	6.351	0.734	0.733	-0.002	0.988	
57	**ES40**	20	0.0 (0.0%)	0	7.0	6.415	0.755	0.751	-0.006	0.886	
mean values		20		0.4	6.6	6.142	0.668	0.723	0.079		

e

No. Population	ID Population	No. Samples	Missing genotypes (%)	N_p	N_a	A_r28	H_o	H_e	F_{IS}	p-value (F_{IS})	
53	**IT24**	20	0.1 (0.5%)	0	6.0	5.411	0.553	0.629	0.124	0.000	***
54	**IT26**	20	0.2 (1.0%)	1	6.0	5.452	0.623	0.659	0.056	0.187	
55	**IT12**	20	0.0 (0.0%)	0	6.3	5.633	0.640	0.638	-0.003	0.951	
56	**RS34**	20	0.4 (2.0%)	0	6.7	5.968	0.629	0.671	0.064	0.141	
mean values		20		0.2	6.3	5.616	0.611	0.649	0.060		

Supplementary File 10 Pairwise populations differentiation (FST) values between the populations analysed

Population 1		Population 2		Fst	p value
1	**IT18**	**2**	**IT22**	0.0049	0.2058
1	**IT18**	**3**	**IT15**	0.0171	0.0120
1	**IT18**	**4**	**IT23**	0.0136	0.0310
1	**IT18**	**5**	**IT64**	0.0411	0.0000
1	**IT18**	**8**	**IT87**	0.0376	0.0000
1	**IT18**	**9**	**IT88**	0.0441	0.0000
1	**IT18**	**10**	**IT89**	0.0411	0.0000
1	**IT18**	**12**	**IT48**	0.0534	0.0000
1	**IT18**	**13**	**IT49**	0.0494	0.0000
1	**IT18**	**14**	**IT50**	0.0413	0.0000
1	**IT18**	**15**	**IT51**	0.0414	0.0000
1	**IT18**	**16**	**IT52**	0.0412	0.0000
1	**IT18**	**17**	**IT53**	0.0448	0.0000
1	**IT18**	**18**	**ES44**	0.0123	0.0549
1	**IT18**	**19**	**ES45**	0.0710	0.0000
1	**IT18**	**20**	**FR08**	0.0301	0.0020
1	**IT18**	**21**	**FR09**	0.0148	0.0180
1	**IT18**	**22**	**FR10**	0.0255	0.0040
1	**IT18**	**23**	**AT01**	0.0178	0.0050
1	**IT18**	**25**	**HR04**	0.0170	0.0070
1	**IT18**	**26**	**HR05**	0.0514	0.0000
1	**IT18**	**27**	**HR07**	0.0184	0.0080
1	**IT18**	**28**	**GR11**	0.0206	0.0090
1	**IT18**	**29**	**CZ30**	0.0070	0.1079
1	**IT18**	**30**	**RS35**	0.0222	0.0020
1	**IT18**	**31**	**IT19**	0.0557	0.0000
1	**IT18**	**32**	**IT20**	0.0859	0.0000
1	**IT18**	**33**	**IT46**	0.0872	0.0000
1	**IT18**	**34**	**IT47**	0.0726	0.0000
1	**IT18**	**35**	**IT17**	0.0760	0.0000
1	**IT18**	**36**	**IT16**	0.0489	0.0000
1	**IT18**	**37**	**ES43**	0.0685	0.0000
1	**IT18**	**38**	**AT02**	0.1123	0.0000
1	**IT18**	**39**	**HR06**	0.0571	0.0000
1	**IT18**	**40**	**CZ31**	0.0837	0.0000
1	**IT18**	**41**	**RO33**	0.0744	0.0000
1	**IT18**	**42**	**RS36**	0.0577	0.0000
1	**IT18**	**43**	**RS37**	0.0366	0.0000
1	**IT18**	**44**	**RS38**	0.0829	0.0000
1	**IT18**	**45**	**RS67**	0.0894	0.0000
1	**IT18**	**46**	**ES41**	0.1558	0.0000
1	**IT18**	**48**	**IT14**	0.1399	0.0000
1	**IT18**	**49**	**IT13**	0.1789	0.0000
1	**IT18**	**51**	**CZ32**	0.1330	0.0000
1	**IT18**	**53**	**IT24**	0.1387	0.0000
1	**IT18**	**54**	**IT26**	0.1176	0.0000
1	**IT18**	**55**	**IT12**	0.1107	0.0000
1	**IT18**	**56**	**RS34**	0.1228	0.0000
1	**IT18**	**57**	**ES40**	0.1200	0.0000
1	**IT18**	**58**	**ES42**	0.0650	0.0000

Population 1		Population 2		Fst	p value
2	**IT22**	**3**	**IT15**	0.0095	0.0669
2	**IT22**	**4**	**IT23**	0.0046	0.1828
2	**IT22**	**5**	**IT64**	0.0451	0.0000
2	**IT22**	**8**	**IT87**	0.0448	0.0000
2	**IT22**	**9**	**IT88**	0.0540	0.0000
2	**IT22**	**10**	**IT89**	0.0348	0.0000
2	**IT22**	**12**	**IT48**	0.0520	0.0000
2	**IT22**	**13**	**IT49**	0.0613	0.0000
2	**IT22**	**14**	**IT50**	0.0376	0.0000
2	**IT22**	**15**	**IT51**	0.0529	0.0000
2	**IT22**	**16**	**IT52**	0.0345	0.0000
2	**IT22**	**17**	**IT53**	0.0408	0.0000
2	**IT22**	**18**	**ES44**	0.0151	0.0250
2	**IT22**	**19**	**ES45**	0.0682	0.0000
2	**IT22**	**20**	**FR08**	0.0230	0.0000
2	**IT22**	**21**	**FR09**	0.0161	0.0040
2	**IT22**	**22**	**FR10**	0.0176	0.0070
2	**IT22**	**23**	**AT01**	0.0210	0.0000
2	**IT22**	**25**	**HR04**	0.0179	0.0040
2	**IT22**	**26**	**HR05**	0.0319	0.0000
2	**IT22**	**27**	**HR07**	0.0143	0.0220
2	**IT22**	**28**	**GR11**	0.0109	0.0559
2	**IT22**	**29**	**CZ30**	0.0049	0.1858
2	**IT22**	**30**	**RS35**	0.0136	0.0250
2	**IT22**	**31**	**IT19**	0.0496	0.0000
2	**IT22**	**32**	**IT20**	0.0904	0.0000
2	**IT22**	**33**	**IT46**	0.0898	0.0000
2	**IT22**	**34**	**IT47**	0.0686	0.0000
2	**IT22**	**35**	**IT17**	0.0829	0.0000
2	**IT22**	**36**	**IT16**	0.0428	0.0000
2	**IT22**	**37**	**ES43**	0.0618	0.0000
2	**IT22**	**38**	**AT02**	0.1229	0.0000
2	**IT22**	**39**	**HR06**	0.0648	0.0000
2	**IT22**	**40**	**CZ31**	0.0904	0.0000
2	**IT22**	**41**	**RO33**	0.0753	0.0000
2	**IT22**	**42**	**RS36**	0.0722	0.0000
2	**IT22**	**43**	**RS37**	0.0377	0.0000
2	**IT22**	**44**	**RS38**	0.0917	0.0000
2	**IT22**	**45**	**RS67**	0.1082	0.0000
2	**IT22**	**46**	**ES41**	0.1071	0.0000
2	**IT22**	**48**	**IT14**	0.1119	0.0000
2	**IT22**	**49**	**IT13**	0.1564	0.0000
2	**IT22**	**51**	**CZ32**	0.1117	0.0000
2	**IT22**	**53**	**IT24**	0.1246	0.0000
2	**IT22**	**54**	**IT26**	0.0923	0.0000
2	**IT22**	**55**	**IT12**	0.0958	0.0000
2	**IT22**	**56**	**RS34**	0.1022	0.0000
2	**IT22**	**57**	**ES40**	0.1039	0.0000
2	**IT22**	**58**	**ES42**	0.0533	0.0000
3	**IT15**	**4**	**IT23**	0.0114	0.0430

Population 1		Population 2		Fst	p value
3	**IT15**	**5**	**IT64**	0.0225	0.0020
3	**IT15**	**8**	**IT87**	0.0381	0.0000
3	**IT15**	**9**	**IT88**	0.0502	0.0000
3	**IT15**	**10**	**IT89**	0.0323	0.0000
3	**IT15**	**12**	**IT48**	0.0362	0.0000
3	**IT15**	**13**	**IT49**	0.0274	0.0020
3	**IT15**	**14**	**IT50**	0.0235	0.0020
3	**IT15**	**15**	**IT51**	0.0368	0.0000
3	**IT15**	**16**	**IT52**	0.0375	0.0000
3	**IT15**	**17**	**IT53**	0.0312	0.0000
3	**IT15**	**18**	**ES44**	0.0221	0.0040
3	**IT15**	**19**	**ES45**	0.0768	0.0000
3	**IT15**	**20**	**FR08**	0.0380	0.0000
3	**IT15**	**21**	**FR09**	0.0257	0.0000
3	**IT15**	**22**	**FR10**	0.0200	0.0090
3	**IT15**	**23**	**AT01**	0.0245	0.0040
3	**IT15**	**25**	**HR04**	0.0229	0.0020
3	**IT15**	**26**	**HR05**	0.0378	0.0000
3	**IT15**	**27**	**HR07**	0.0113	0.0470
3	**IT15**	**28**	**GR11**	0.0158	0.0210
3	**IT15**	**29**	**CZ30**	0.0091	0.0679
3	**IT15**	**30**	**RS35**	0.0212	0.0040
3	**IT15**	**31**	**IT19**	0.0599	0.0000
3	**IT15**	**32**	**IT20**	0.1050	0.0000
3	**IT15**	**33**	**IT46**	0.0839	0.0000
3	**IT15**	**34**	**IT47**	0.0669	0.0000
3	**IT15**	**35**	**IT17**	0.0921	0.0000
3	**IT15**	**36**	**IT16**	0.0603	0.0000
3	**IT15**	**37**	**ES43**	0.0587	0.0000
3	**IT15**	**38**	**AT02**	0.1201	0.0000
3	**IT15**	**39**	**HR06**	0.0770	0.0000
3	**IT15**	**40**	**CZ31**	0.0897	0.0000
3	**IT15**	**41**	**RO33**	0.1011	0.0000
3	**IT15**	**42**	**RS36**	0.0856	0.0000
3	**IT15**	**43**	**RS37**	0.0611	0.0000
3	**IT15**	**44**	**RS38**	0.1279	0.0000
3	**IT15**	**45**	**RS67**	0.1189	0.0000
3	**IT15**	**46**	**ES41**	0.1441	0.0000
3	**IT15**	**48**	**IT14**	0.1406	0.0000
3	**IT15**	**49**	**IT13**	0.1883	0.0000
3	**IT15**	**51**	**CZ32**	0.1454	0.0000
3	**IT15**	**53**	**IT24**	0.1309	0.0000
3	**IT15**	**54**	**IT26**	0.1146	0.0000
3	**IT15**	**55**	**IT12**	0.1125	0.0000
3	**IT15**	**56**	**RS34**	0.1166	0.0000
3	**IT15**	**57**	**ES40**	0.1253	0.0000
3	**IT15**	**58**	**ES42**	0.0755	0.0000
4	**IT23**	**5**	**IT64**	0.0334	0.0000
4	**IT23**	**8**	**IT87**	0.0319	0.0000
4	**IT23**	**9**	**IT88**	0.0442	0.0000

Population 1		Population 2		Fst	p value
4	**IT23**	**10**	**IT89**	0.0319	0.0000
4	**IT23**	**12**	**IT48**	0.0385	0.0000
4	**IT23**	**13**	**IT49**	0.0438	0.0000
4	**IT23**	**14**	**IT50**	0.0409	0.0000
4	**IT23**	**15**	**IT51**	0.0495	0.0000
4	**IT23**	**16**	**IT52**	0.0330	0.0000
4	**IT23**	**17**	**IT53**	0.0401	0.0000
4	**IT23**	**18**	**ES44**	0.0266	0.0020
4	**IT23**	**19**	**ES45**	0.0840	0.0000
4	**IT23**	**20**	**FR08**	0.0390	0.0000
4	**IT23**	**21**	**FR09**	0.0412	0.0000
4	**IT23**	**22**	**FR10**	0.0317	0.0000
4	**IT23**	**23**	**AT01**	0.0188	0.0020
4	**IT23**	**25**	**HR04**	0.0300	0.0000
4	**IT23**	**26**	**HR05**	0.0483	0.0000
4	**IT23**	**27**	**HR07**	0.0044	0.2228
4	**IT23**	**28**	**GR11**	0.0195	0.0090
4	**IT23**	**29**	**CZ30**	0.0022	0.3526
4	**IT23**	**30**	**RS35**	0.0277	0.0000
4	**IT23**	**31**	**IT19**	0.0863	0.0000
4	**IT23**	**32**	**IT20**	0.1276	0.0000
4	**IT23**	**33**	**IT46**	0.1082	0.0000
4	**IT23**	**34**	**IT47**	0.0787	0.0000
4	**IT23**	**35**	**IT17**	0.0760	0.0000
4	**IT23**	**36**	**IT16**	0.0365	0.0000
4	**IT23**	**37**	**ES43**	0.0869	0.0000
4	**IT23**	**38**	**AT02**	0.1391	0.0000
4	**IT23**	**39**	**HR06**	0.0817	0.0000
4	**IT23**	**40**	**CZ31**	0.1124	0.0000
4	**IT23**	**41**	**RO33**	0.0909	0.0000
4	**IT23**	**42**	**RS36**	0.0857	0.0000
4	**IT23**	**43**	**RS37**	0.0498	0.0000
4	**IT23**	**44**	**RS38**	0.1017	0.0000
4	**IT23**	**45**	**RS67**	0.1261	0.0000
4	**IT23**	**46**	**ES41**	0.1402	0.0000
4	**IT23**	**48**	**IT14**	0.1457	0.0000
4	**IT23**	**49**	**IT13**	0.1768	0.0000
4	**IT23**	**51**	**CZ32**	0.1410	0.0000
4	**IT23**	**53**	**IT24**	0.1248	0.0000
4	**IT23**	**54**	**IT26**	0.1031	0.0000
4	**IT23**	**55**	**IT12**	0.0925	0.0000
4	**IT23**	**56**	**RS34**	0.1173	0.0000
4	**IT23**	**57**	**ES40**	0.1220	0.0000
4	**IT23**	**58**	**ES42**	0.0811	0.0000
5	**IT64**	**8**	**IT87**	0.0041	0.2697
5	**IT64**	**9**	**IT88**	0.0056	0.2088
5	**IT64**	**10**	**IT89**	0.0121	0.0480
5	**IT64**	**12**	**IT48**	0.0231	0.0020
5	**IT64**	**13**	**IT49**	0.0136	0.0240
5	**IT64**	**14**	**IT50**	0.0148	0.0080

Population 1		Population 2		Fst	p value
5	**IT64**	**15**	**IT51**	0.0419	0.0000
5	**IT64**	**16**	**IT52**	0.0333	0.0000
5	**IT64**	**17**	**IT53**	0.0278	0.0000
5	**IT64**	**18**	**ES44**	0.0373	0.0000
5	**IT64**	**19**	**ES45**	0.0602	0.0000
5	**IT64**	**20**	**FR08**	0.0657	0.0000
5	**IT64**	**21**	**FR09**	0.0576	0.0000
5	**IT64**	**22**	**FR10**	0.0515	0.0000
5	**IT64**	**23**	**AT01**	0.0358	0.0000
5	**IT64**	**25**	**HR04**	0.0603	0.0000
5	**IT64**	**26**	**HR05**	0.0708	0.0000
5	**IT64**	**27**	**HR07**	0.0321	0.0000
5	**IT64**	**28**	**GR11**	0.0340	0.0000
5	**IT64**	**29**	**CZ30**	0.0375	0.0000
5	**IT64**	**30**	**RS35**	0.0532	0.0000
5	**IT64**	**31**	**IT19**	0.1094	0.0000
5	**IT64**	**32**	**IT20**	0.1393	0.0000
5	**IT64**	**33**	**IT46**	0.0808	0.0000
5	**IT64**	**34**	**IT47**	0.0617	0.0000
5	**IT64**	**35**	**IT17**	0.1107	0.0000
5	**IT64**	**36**	**IT16**	0.0765	0.0000
5	**IT64**	**37**	**ES43**	0.1122	0.0000
5	**IT64**	**38**	**AT02**	0.1483	0.0000
5	**IT64**	**39**	**HR06**	0.1056	0.0000
5	**IT64**	**40**	**CZ31**	0.1265	0.0000
5	**IT64**	**41**	**RO33**	0.1104	0.0000
5	**IT64**	**42**	**RS36**	0.1166	0.0000
5	**IT64**	**43**	**RS37**	0.0768	0.0000
5	**IT64**	**44**	**RS38**	0.1302	0.0000
5	**IT64**	**45**	**RS67**	0.1112	0.0000
5	**IT64**	**46**	**ES41**	0.1758	0.0000
5	**IT64**	**48**	**IT14**	0.1801	0.0000
5	**IT64**	**49**	**IT13**	0.2134	0.0000
5	**IT64**	**51**	**CZ32**	0.1721	0.0000
5	**IT64**	**53**	**IT24**	0.1496	0.0000
5	**IT64**	**54**	**IT26**	0.1389	0.0000
5	**IT64**	**55**	**IT12**	0.1365	0.0000
5	**IT64**	**56**	**RS34**	0.1470	0.0000
5	**IT64**	**57**	**ES40**	0.1437	0.0000
5	**IT64**	**58**	**ES42**	0.1022	0.0000
8	**IT87**	**9**	**IT88**	0.0143	0.0290
8	**IT87**	**10**	**IT89**	0.0194	0.0100
8	**IT87**	**12**	**IT48**	0.0251	0.0000
8	**IT87**	**13**	**IT49**	0.0303	0.0000
8	**IT87**	**14**	**IT50**	0.0143	0.0100
8	**IT87**	**15**	**IT51**	0.0379	0.0000
8	**IT87**	**16**	**IT52**	0.0261	0.0030
8	**IT87**	**17**	**IT53**	0.0243	0.0020
8	**IT87**	**18**	**ES44**	0.0415	0.0000
8	**IT87**	**19**	**ES45**	0.0556	0.0000

Population 1		Population 2		Fst	p value
8	**IT87**	**20**	**FR08**	0.0560	0.0000
8	**IT87**	**21**	**FR09**	0.0467	0.0000
8	**IT87**	**22**	**FR10**	0.0546	0.0000
8	**IT87**	**23**	**AT01**	0.0379	0.0000
8	**IT87**	**25**	**HR04**	0.0632	0.0000
8	**IT87**	**26**	**HR05**	0.0734	0.0000
8	**IT87**	**27**	**HR07**	0.0273	0.0000
8	**IT87**	**28**	**GR11**	0.0355	0.0000
8	**IT87**	**29**	**CZ30**	0.0409	0.0000
8	**IT87**	**30**	**RS35**	0.0538	0.0000
8	**IT87**	**31**	**IT19**	0.1041	0.0000
8	**IT87**	**32**	**IT20**	0.1325	0.0000
8	**IT87**	**33**	**IT46**	0.0856	0.0000
8	**IT87**	**34**	**IT47**	0.0564	0.0000
8	**IT87**	**35**	**IT17**	0.0984	0.0000
8	**IT87**	**36**	**IT16**	0.0625	0.0000
8	**IT87**	**37**	**ES43**	0.0951	0.0000
8	**IT87**	**38**	**AT02**	0.1386	0.0000
8	**IT87**	**39**	**HR06**	0.0839	0.0000
8	**IT87**	**40**	**CZ31**	0.1166	0.0000
8	**IT87**	**41**	**RO33**	0.0905	0.0000
8	**IT87**	**42**	**RS36**	0.0972	0.0000
8	**IT87**	**43**	**RS37**	0.0604	0.0000
8	**IT87**	**44**	**RS38**	0.0990	0.0000
8	**IT87**	**45**	**RS67**	0.1097	0.0000
8	**IT87**	**46**	**ES41**	0.1532	0.0000
8	**IT87**	**48**	**IT14**	0.1503	0.0000
8	**IT87**	**49**	**IT13**	0.1686	0.0000
8	**IT87**	**51**	**CZ32**	0.1515	0.0000
8	**IT87**	**53**	**IT24**	0.1240	0.0000
8	**IT87**	**54**	**IT26**	0.1136	0.0000
8	**IT87**	**55**	**IT12**	0.1061	0.0000
8	**IT87**	**56**	**RS34**	0.1288	0.0000
8	**IT87**	**57**	**ES40**	0.1222	0.0000
8	**IT87**	**58**	**ES42**	0.0964	0.0000
9	**IT88**	**10**	**IT89**	0.0158	0.0300
9	**IT88**	**12**	**IT48**	0.0287	0.0000
9	**IT88**	**13**	**IT49**	0.0309	0.0000
9	**IT88**	**14**	**IT50**	0.0240	0.0000
9	**IT88**	**15**	**IT51**	0.0506	0.0000
9	**IT88**	**16**	**IT52**	0.0325	0.0000
9	**IT88**	**17**	**IT53**	0.0318	0.0000
9	**IT88**	**18**	**ES44**	0.0462	0.0000
9	**IT88**	**19**	**ES45**	0.0501	0.0000
9	**IT88**	**20**	**FR08**	0.0660	0.0000
9	**IT88**	**21**	**FR09**	0.0604	0.0000
9	**IT88**	**22**	**FR10**	0.0552	0.0000
9	**IT88**	**23**	**AT01**	0.0371	0.0000
9	**IT88**	**25**	**HR04**	0.0524	0.0000
9	**IT88**	**26**	**HR05**	0.0729	0.0000

Population 1		Population 2		Fst	p value
9	IT88	27	HR07	0.0311	0.0000
9	IT88	28	GR11	0.0406	0.0020
9	IT88	29	CZ30	0.0431	0.0000
9	IT88	30	RS35	0.0613	0.0000
9	IT88	31	IT19	0.1091	0.0000
9	IT88	32	IT20	0.1347	0.0000
9	IT88	33	IT46	0.0905	0.0000
9	IT88	34	IT47	0.0695	0.0000
9	IT88	35	IT17	0.1126	0.0000
9	IT88	36	IT16	0.0777	0.0000
9	IT88	37	ES43	0.1183	0.0000
9	IT88	38	AT02	0.1418	0.0000
9	IT88	39	HR06	0.0971	0.0000
9	IT88	40	CZ31	0.1305	0.0000
9	IT88	41	RO33	0.1073	0.0000
9	IT88	42	RS36	0.1079	0.0000
9	IT88	43	RS37	0.0736	0.0000
9	IT88	44	RS38	0.1334	0.0000
9	IT88	45	RS67	0.1101	0.0000
9	IT88	46	ES41	0.1771	0.0000
9	IT88	48	IT14	0.1819	0.0000
9	IT88	49	IT13	0.1872	0.0000
9	IT88	51	CZ32	0.1666	0.0000
9	IT88	53	IT24	0.1482	0.0000
9	IT88	54	IT26	0.1359	0.0000
9	IT88	55	IT12	0.1329	0.0000
9	IT88	56	RS34	0.1429	0.0000
9	IT88	57	ES40	0.1264	0.0000
9	IT88	58	ES42	0.1060	0.0000
10	IT89	12	IT48	0.0322	0.0000
10	IT89	13	IT49	0.0409	0.0000
10	IT89	14	IT50	0.0176	0.0070
10	IT89	15	IT51	0.0423	0.0000
10	IT89	16	IT52	0.0354	0.0000
10	IT89	17	IT53	0.0245	0.0020
10	IT89	18	ES44	0.0433	0.0000
10	IT89	19	ES45	0.0660	0.0000
10	IT89	20	FR08	0.0634	0.0000
10	IT89	21	FR09	0.0584	0.0000
10	IT89	22	FR10	0.0526	0.0000
10	IT89	23	AT01	0.0350	0.0000
10	IT89	25	HR04	0.0560	0.0000
10	IT89	26	HR05	0.0513	0.0000
10	IT89	27	HR07	0.0314	0.0000
10	IT89	28	GR11	0.0296	0.0000
10	IT89	29	CZ30	0.0415	0.0000
10	IT89	30	RS35	0.0494	0.0000
10	IT89	31	IT19	0.0935	0.0000
10	IT89	32	IT20	0.1273	0.0000
10	IT89	33	IT46	0.0946	0.0000

Population 1		Population 2		Fst	p value
10	IT89	34	IT47	0.0700	0.0000
10	IT89	35	IT17	0.1051	0.0000
10	IT89	36	IT16	0.0716	0.0000
10	IT89	37	ES43	0.1071	0.0000
10	IT89	38	AT02	0.1418	0.0000
10	IT89	39	HR06	0.0877	0.0000
10	IT89	40	CZ31	0.1247	0.0000
10	IT89	41	RO33	0.0941	0.0000
10	IT89	42	RS36	0.1004	0.0000
10	IT89	43	RS37	0.0635	0.0000
10	IT89	44	RS38	0.1215	0.0000
10	IT89	45	RS67	0.1039	0.0000
10	IT89	46	ES41	0.1638	0.0000
10	IT89	48	IT14	0.1573	0.0000
10	IT89	49	IT13	0.1756	0.0000
10	IT89	51	CZ32	0.1566	0.0000
10	IT89	53	IT24	0.1286	0.0000
10	IT89	54	IT26	0.1184	0.0000
10	IT89	55	IT12	0.1164	0.0000
10	IT89	56	RS34	0.1267	0.0000
10	IT89	57	ES40	0.1332	0.0000
10	IT89	58	ES42	0.1021	0.0000
12	IT48	13	IT49	0.0222	0.0000
12	IT48	14	IT50	0.0209	0.0020
12	IT48	15	IT51	0.0246	0.0000
12	IT48	16	IT52	0.0189	0.0040
12	IT48	17	IT53	0.0305	0.0000
12	IT48	18	ES44	0.0400	0.0000
12	IT48	19	ES45	0.0523	0.0000
12	IT48	20	FR08	0.0618	0.0000
12	IT48	21	FR09	0.0470	0.0000
12	IT48	22	FR10	0.0539	0.0000
12	IT48	23	AT01	0.0476	0.0000
12	IT48	25	HR04	0.0442	0.0000
12	IT48	26	HR05	0.0541	0.0000
12	IT48	27	HR07	0.0359	0.0000
12	IT48	28	GR11	0.0275	0.0000
12	IT48	29	CZ30	0.0339	0.0000
12	IT48	30	RS35	0.0559	0.0000
12	IT48	31	IT19	0.0960	0.0000
12	IT48	32	IT20	0.1345	0.0000
12	IT48	33	IT46	0.0758	0.0000
12	IT48	34	IT47	0.0628	0.0000
12	IT48	35	IT17	0.1029	0.0000
12	IT48	36	IT16	0.0736	0.0000
12	IT48	37	ES43	0.0954	0.0000
12	IT48	38	AT02	0.1389	0.0000
12	IT48	39	HR06	0.0873	0.0000
12	IT48	40	CZ31	0.1161	0.0000
12	IT48	41	RO33	0.0970	0.0000

Population 1		Population 2		Fst	p value
12	**IT48**	**42**	**RS36**	0.0997	0.0000
12	**IT48**	**43**	**RS37**	0.0768	0.0000
12	**IT48**	**44**	**RS38**	0.1250	0.0000
12	**IT48**	**45**	**RS67**	0.1142	0.0000
12	**IT48**	**46**	**ES41**	0.1429	0.0000
12	**IT48**	**48**	**IT14**	0.1578	0.0000
12	**IT48**	**49**	**IT13**	0.1626	0.0000
12	**IT48**	**51**	**CZ32**	0.1507	0.0000
12	**IT48**	**53**	**IT24**	0.1322	0.0000
12	**IT48**	**54**	**IT26**	0.1272	0.0000
12	**IT48**	**55**	**IT12**	0.1237	0.0000
12	**IT48**	**56**	**RS34**	0.1235	0.0000
12	**IT48**	**57**	**ES40**	0.1092	0.0000
12	**IT48**	**58**	**ES42**	0.0993	0.0000
13	**IT49**	**14**	**IT50**	0.0261	0.0020
13	**IT49**	**15**	**IT51**	0.0355	0.0020
13	**IT49**	**16**	**IT52**	0.0370	0.0000
13	**IT49**	**17**	**IT53**	0.0305	0.0000
13	**IT49**	**18**	**ES44**	0.0499	0.0000
13	**IT49**	**19**	**ES45**	0.0770	0.0000
13	**IT49**	**20**	**FR08**	0.0731	0.0000
13	**IT49**	**21**	**FR09**	0.0621	0.0000
13	**IT49**	**22**	**FR10**	0.0587	0.0000
13	**IT49**	**23**	**AT01**	0.0502	0.0000
13	**IT49**	**25**	**HR04**	0.0597	0.0000
13	**IT49**	**26**	**HR05**	0.0678	0.0000
13	**IT49**	**27**	**HR07**	0.0397	0.0000
13	**IT49**	**28**	**GR11**	0.0399	0.0000
13	**IT49**	**29**	**CZ30**	0.0313	0.0000
13	**IT49**	**30**	**RS35**	0.0586	0.0000
13	**IT49**	**31**	**IT19**	0.1027	0.0000
13	**IT49**	**32**	**IT20**	0.1465	0.0000
13	**IT49**	**33**	**IT46**	0.0963	0.0000
13	**IT49**	**34**	**IT47**	0.0798	0.0000
13	**IT49**	**35**	**IT17**	0.1176	0.0000
13	**IT49**	**36**	**IT16**	0.0813	0.0000
13	**IT49**	**37**	**ES43**	0.1007	0.0000
13	**IT49**	**38**	**AT02**	0.1565	0.0000
13	**IT49**	**39**	**HR06**	0.0996	0.0000
13	**IT49**	**40**	**CZ31**	0.1244	0.0000
13	**IT49**	**41**	**RO33**	0.1224	0.0000
13	**IT49**	**42**	**RS36**	0.1200	0.0000
13	**IT49**	**43**	**RS37**	0.0972	0.0000
13	**IT49**	**44**	**RS38**	0.1512	0.0000
13	**IT49**	**45**	**RS67**	0.1340	0.0000
13	**IT49**	**46**	**ES41**	0.1871	0.0000
13	**IT49**	**48**	**IT14**	0.1870	0.0000
13	**IT49**	**49**	**IT13**	0.2155	0.0000
13	**IT49**	**51**	**CZ32**	0.1749	0.0000
13	**IT49**	**53**	**IT24**	0.1641	0.0000
13	**IT49**	**54**	**IT26**	0.1549	0.0000
13	**IT49**	**55**	**IT12**	0.1424	0.0000
13	**IT49**	**56**	**RS34**	0.1625	0.0000
13	**IT49**	**57**	**ES40**	0.1364	0.0000
13	**IT49**	**58**	**ES42**	0.1107	0.0000
14	**IT50**	**15**	**IT51**	0.0209	0.0040
14	**IT50**	**16**	**IT52**	0.0198	0.0030
14	**IT50**	**17**	**IT53**	0.0156	0.0040
14	**IT50**	**18**	**ES44**	0.0258	0.0000
14	**IT50**	**19**	**ES45**	0.0432	0.0000
14	**IT50**	**20**	**FR08**	0.0475	0.0000
14	**IT50**	**21**	**FR09**	0.0369	0.0000
14	**IT50**	**22**	**FR10**	0.0305	0.0000
14	**IT50**	**23**	**AT01**	0.0400	0.0000
14	**IT50**	**25**	**HR04**	0.0396	0.0000
14	**IT50**	**26**	**HR05**	0.0478	0.0000
14	**IT50**	**27**	**HR07**	0.0239	0.0000
14	**IT50**	**28**	**GR11**	0.0251	0.0030
14	**IT50**	**29**	**CZ30**	0.0351	0.0000
14	**IT50**	**30**	**RS35**	0.0423	0.0000
14	**IT50**	**31**	**IT19**	0.0849	0.0000
14	**IT50**	**32**	**IT20**	0.1240	0.0000
14	**IT50**	**33**	**IT46**	0.0788	0.0000
14	**IT50**	**34**	**IT47**	0.0588	0.0000
14	**IT50**	**35**	**IT17**	0.1162	0.0000
14	**IT50**	**36**	**IT16**	0.0675	0.0000
14	**IT50**	**37**	**ES43**	0.0876	0.0000
14	**IT50**	**38**	**AT02**	0.1277	0.0000
14	**IT50**	**39**	**HR06**	0.0866	0.0000
14	**IT50**	**40**	**CZ31**	0.1021	0.0000
14	**IT50**	**41**	**RO33**	0.1035	0.0000
14	**IT50**	**42**	**RS36**	0.0967	0.0000
14	**IT50**	**43**	**RS37**	0.0725	0.0000
14	**IT50**	**44**	**RS38**	0.1335	0.0000
14	**IT50**	**45**	**RS67**	0.1056	0.0000
14	**IT50**	**46**	**ES41**	0.1476	0.0000
14	**IT50**	**48**	**IT14**	0.1551	0.0000
14	**IT50**	**49**	**IT13**	0.1850	0.0000
14	**IT50**	**51**	**CZ32**	0.1478	0.0000
14	**IT50**	**53**	**IT24**	0.1329	0.0000
14	**IT50**	**54**	**IT26**	0.1299	0.0000
14	**IT50**	**55**	**IT12**	0.1228	0.0000
14	**IT50**	**56**	**RS34**	0.1255	0.0000
14	**IT50**	**57**	**ES40**	0.1232	0.0000
14	**IT50**	**58**	**ES42**	0.0852	0.0000
15	**IT51**	**16**	**IT52**	0.0184	0.0080
15	**IT51**	**17**	**IT53**	0.0296	0.0000
15	**IT51**	**18**	**ES44**	0.0354	0.0000
15	**IT51**	**19**	**ES45**	0.0443	0.0000
15	**IT51**	**20**	**FR08**	0.0489	0.0000

Population 1		Population 2		Fst	p value
15	**IT51**	**21**	**FR09**	0.0368	0.0000
15	**IT51**	**22**	**FR10**	0.0466	0.0000
15	**IT51**	**23**	**AT01**	0.0531	0.0000
15	**IT51**	**25**	**HR04**	0.0328	0.0000
15	**IT51**	**26**	**HR05**	0.0488	0.0000
15	**IT51**	**27**	**HR07**	0.0428	0.0000
15	**IT51**	**28**	**GR11**	0.0326	0.0000
15	**IT51**	**29**	**CZ30**	0.0281	0.0000
15	**IT51**	**30**	**RS35**	0.0419	0.0000
15	**IT51**	**31**	**IT19**	0.0717	0.0000
15	**IT51**	**32**	**IT20**	0.1008	0.0000
15	**IT51**	**33**	**IT46**	0.0688	0.0000
15	**IT51**	**34**	**IT47**	0.0488	0.0000
15	**IT51**	**35**	**IT17**	0.0873	0.0000
15	**IT51**	**36**	**IT16**	0.0671	0.0000
15	**IT51**	**37**	**ES43**	0.0783	0.0000
15	**IT51**	**38**	**AT02**	0.1124	0.0000
15	**IT51**	**39**	**HR06**	0.0605	0.0000
15	**IT51**	**40**	**CZ31**	0.0825	0.0000
15	**IT51**	**41**	**RO33**	0.0746	0.0000
15	**IT51**	**42**	**RS36**	0.0623	0.0000
15	**IT51**	**43**	**RS37**	0.0598	0.0000
15	**IT51**	**44**	**RS38**	0.1049	0.0000
15	**IT51**	**45**	**RS67**	0.0826	0.0000
15	**IT51**	**46**	**ES41**	0.1475	0.0000
15	**IT51**	**48**	**IT14**	0.1318	0.0000
15	**IT51**	**49**	**IT13**	0.1774	0.0000
15	**IT51**	**51**	**CZ32**	0.1282	0.0000
15	**IT51**	**53**	**IT24**	0.1277	0.0000
15	**IT51**	**54**	**IT26**	0.1296	0.0000
15	**IT51**	**55**	**IT12**	0.1280	0.0000
15	**IT51**	**56**	**RS34**	0.1187	0.0000
15	**IT51**	**57**	**ES40**	0.1169	0.0000
15	**IT51**	**58**	**ES42**	0.0909	0.0000
16	**IT52**	**17**	**IT53**	0.0093	0.0669
16	**IT52**	**18**	**ES44**	0.0281	0.0000
16	**IT52**	**19**	**ES45**	0.0433	0.0000
16	**IT52**	**20**	**FR08**	0.0422	0.0000
16	**IT52**	**21**	**FR09**	0.0318	0.0000
16	**IT52**	**22**	**FR10**	0.0291	0.0000
16	**IT52**	**23**	**AT01**	0.0447	0.0000
16	**IT52**	**25**	**HR04**	0.0339	0.0000
16	**IT52**	**26**	**HR05**	0.0504	0.0000
16	**IT52**	**27**	**HR07**	0.0388	0.0000
16	**IT52**	**28**	**GR11**	0.0387	0.0000
16	**IT52**	**29**	**CZ30**	0.0287	0.0000
16	**IT52**	**30**	**RS35**	0.0353	0.0000
16	**IT52**	**31**	**IT19**	0.0700	0.0000
16	**IT52**	**32**	**IT20**	0.1027	0.0000
16	**IT52**	**33**	**IT46**	0.0675	0.0000
16	**IT52**	**34**	**IT47**	0.0476	0.0000
16	**IT52**	**35**	**IT17**	0.1078	0.0000
16	**IT52**	**36**	**IT16**	0.0587	0.0000
16	**IT52**	**37**	**ES43**	0.0884	0.0000
16	**IT52**	**38**	**AT02**	0.1301	0.0000
16	**IT52**	**39**	**HR06**	0.0785	0.0000
16	**IT52**	**40**	**CZ31**	0.0935	0.0000
16	**IT52**	**41**	**RO33**	0.0858	0.0000
16	**IT52**	**42**	**RS36**	0.0799	0.0000
16	**IT52**	**43**	**RS37**	0.0599	0.0000
16	**IT52**	**44**	**RS38**	0.1167	0.0000
16	**IT52**	**45**	**RS67**	0.0819	0.0000
16	**IT52**	**46**	**ES41**	0.1350	0.0000
16	**IT52**	**48**	**IT14**	0.1363	0.0000
16	**IT52**	**49**	**IT13**	0.1539	0.0000
16	**IT52**	**51**	**CZ32**	0.1297	0.0000
16	**IT52**	**53**	**IT24**	0.1346	0.0000
16	**IT52**	**54**	**IT26**	0.1201	0.0000
16	**IT52**	**55**	**IT12**	0.1304	0.0000
16	**IT52**	**56**	**RS34**	0.1292	0.0000
16	**IT52**	**57**	**ES40**	0.1059	0.0000
16	**IT52**	**58**	**ES42**	0.0722	0.0000
17	**IT53**	**18**	**ES44**	0.0259	0.0000
17	**IT53**	**19**	**ES45**	0.0474	0.0000
17	**IT53**	**20**	**FR08**	0.0447	0.0000
17	**IT53**	**21**	**FR09**	0.0391	0.0000
17	**IT53**	**22**	**FR10**	0.0333	0.0000
17	**IT53**	**23**	**AT01**	0.0352	0.0000
17	**IT53**	**25**	**HR04**	0.0493	0.0000
17	**IT53**	**26**	**HR05**	0.0634	0.0000
17	**IT53**	**27**	**HR07**	0.0338	0.0000
17	**IT53**	**28**	**GR11**	0.0308	0.0000
17	**IT53**	**29**	**CZ30**	0.0301	0.0000
17	**IT53**	**30**	**RS35**	0.0347	0.0000
17	**IT53**	**31**	**IT19**	0.0825	0.0000
17	**IT53**	**32**	**IT20**	0.1142	0.0000
17	**IT53**	**33**	**IT46**	0.0747	0.0000
17	**IT53**	**34**	**IT47**	0.0512	0.0000
17	**IT53**	**35**	**IT17**	0.1113	0.0000
17	**IT53**	**36**	**IT16**	0.0592	0.0000
17	**IT53**	**37**	**ES43**	0.0890	0.0000
17	**IT53**	**38**	**AT02**	0.1395	0.0000
17	**IT53**	**39**	**HR06**	0.0869	0.0000
17	**IT53**	**40**	**CZ31**	0.0976	0.0000
17	**IT53**	**41**	**RO33**	0.1024	0.0000
17	**IT53**	**42**	**RS36**	0.0943	0.0000
17	**IT53**	**43**	**RS37**	0.0702	0.0000
17	**IT53**	**44**	**RS38**	0.1306	0.0000
17	**IT53**	**45**	**RS67**	0.1055	0.0000
17	**IT53**	**46**	**ES41**	0.1435	0.0000

Population 1		Population 2		Fst	p value
17	**IT53**	**48**	**IT14**	0.1414	0.0000
17	**IT53**	**49**	**IT13**	0.1741	0.0000
17	**IT53**	**51**	**CZ32**	0.1365	0.0000
17	**IT53**	**53**	**IT24**	0.1359	0.0000
17	**IT53**	**54**	**IT26**	0.1239	0.0000
17	**IT53**	**55**	**IT12**	0.1244	0.0000
17	**IT53**	**56**	**RS34**	0.1314	0.0000
17	**IT53**	**57**	**ES40**	0.1161	0.0000
17	**IT53**	**58**	**ES42**	0.0871	0.0000
18	**ES44**	**19**	**ES45**	0.0127	0.0210
18	**ES44**	**20**	**FR08**	0.0077	0.1139
18	**ES44**	**21**	**FR09**	0.0021	0.3297
18	**ES44**	**22**	**FR10**	0.0089	0.0909
18	**ES44**	**23**	**AT01**	0.0295	0.0000
18	**ES44**	**25**	**HR04**	0.0206	0.0050
18	**ES44**	**26**	**HR05**	0.0468	0.0000
18	**ES44**	**27**	**HR07**	0.0176	0.0150
18	**ES44**	**28**	**GR11**	0.0187	0.0160
18	**ES44**	**29**	**CZ30**	0.0099	0.0490
18	**ES44**	**30**	**RS35**	0.0211	0.0050
18	**ES44**	**31**	**IT19**	0.0468	0.0000
18	**ES44**	**32**	**IT20**	0.0613	0.0000
18	**ES44**	**33**	**IT46**	0.0574	0.0000
18	**ES44**	**34**	**IT47**	0.0418	0.0000
18	**ES44**	**35**	**IT17**	0.0719	0.0000
18	**ES44**	**36**	**IT16**	0.0338	0.0000
18	**ES44**	**37**	**ES43**	0.0666	0.0000
18	**ES44**	**38**	**AT02**	0.0922	0.0000
18	**ES44**	**39**	**HR06**	0.0502	0.0000
18	**ES44**	**40**	**CZ31**	0.0575	0.0000
18	**ES44**	**41**	**RO33**	0.0602	0.0000
18	**ES44**	**42**	**RS36**	0.0545	0.0000
18	**ES44**	**43**	**RS37**	0.0253	0.0000
18	**ES44**	**44**	**RS38**	0.0796	0.0000
18	**ES44**	**45**	**RS67**	0.0687	0.0000
18	**ES44**	**46**	**ES41**	0.1078	0.0000
18	**ES44**	**48**	**IT14**	0.1088	0.0000
18	**ES44**	**49**	**IT13**	0.1487	0.0000
18	**ES44**	**51**	**CZ32**	0.1025	0.0000
18	**ES44**	**53**	**IT24**	0.1236	0.0000
18	**ES44**	**54**	**IT26**	0.1069	0.0000
18	**ES44**	**55**	**IT12**	0.1099	0.0000
18	**ES44**	**56**	**RS34**	0.1023	0.0000
18	**ES44**	**57**	**ES40**	0.0808	0.0000
18	**ES44**	**58**	**ES42**	0.0293	0.0030
19	**ES45**	**20**	**FR08**	0.0332	0.0000
19	**ES45**	**21**	**FR09**	0.0290	0.0000
19	**ES45**	**22**	**FR10**	0.0496	0.0000
19	**ES45**	**23**	**AT01**	0.0621	0.0000
19	**ES45**	**25**	**HR04**	0.0495	0.0000
19	**ES45**	**26**	**HR05**	0.0636	0.0000
19	**ES45**	**27**	**HR07**	0.0594	0.0000
19	**ES45**	**28**	**GR11**	0.0385	0.0000
19	**ES45**	**29**	**CZ30**	0.0570	0.0000
19	**ES45**	**30**	**RS35**	0.0622	0.0000
19	**ES45**	**31**	**IT19**	0.0684	0.0000
19	**ES45**	**32**	**IT20**	0.0683	0.0000
19	**ES45**	**33**	**IT46**	0.0348	0.0000
19	**ES45**	**34**	**IT47**	0.0274	0.0000
19	**ES45**	**35**	**IT17**	0.0787	0.0000
19	**ES45**	**36**	**IT16**	0.0537	0.0000
19	**ES45**	**37**	**ES43**	0.0848	0.0000
19	**ES45**	**38**	**AT02**	0.0795	0.0000
19	**ES45**	**39**	**HR06**	0.0544	0.0000
19	**ES45**	**40**	**CZ31**	0.0640	0.0000
19	**ES45**	**41**	**RO33**	0.0512	0.0000
19	**ES45**	**42**	**RS36**	0.0525	0.0000
19	**ES45**	**43**	**RS37**	0.0485	0.0000
19	**ES45**	**44**	**RS38**	0.0818	0.0000
19	**ES45**	**45**	**RS67**	0.0455	0.0000
19	**ES45**	**46**	**ES41**	0.1117	0.0000
19	**ES45**	**48**	**IT14**	0.1122	0.0000
19	**ES45**	**49**	**IT13**	0.1369	0.0000
19	**ES45**	**51**	**CZ32**	0.1075	0.0000
19	**ES45**	**53**	**IT24**	0.1414	0.0000
19	**ES45**	**54**	**IT26**	0.1342	0.0000
19	**ES45**	**55**	**IT12**	0.1493	0.0000
19	**ES45**	**56**	**RS34**	0.1210	0.0000
19	**ES45**	**57**	**ES40**	0.0792	0.0000
19	**ES45**	**58**	**ES42**	0.0458	0.0000
20	**FR08**	**21**	**FR09**	0.0006	0.4196
20	**FR08**	**22**	**FR10**	-0.0044	0.7173
20	**FR08**	**23**	**AT01**	0.0388	0.0000
20	**FR08**	**25**	**HR04**	0.0216	0.0000
20	**FR08**	**26**	**HR05**	0.0339	0.0000
20	**FR08**	**27**	**HR07**	0.0251	0.0020
20	**FR08**	**28**	**GR11**	0.0229	0.0040
20	**FR08**	**29**	**CZ30**	0.0247	0.0000
20	**FR08**	**30**	**RS35**	0.0364	0.0000
20	**FR08**	**31**	**IT19**	0.0449	0.0000
20	**FR08**	**32**	**IT20**	0.0623	0.0000
20	**FR08**	**33**	**IT46**	0.0685	0.0000
20	**FR08**	**34**	**IT47**	0.0521	0.0000
20	**FR08**	**35**	**IT17**	0.0546	0.0000
20	**FR08**	**36**	**IT16**	0.0283	0.0000
20	**FR08**	**37**	**ES43**	0.0422	0.0000
20	**FR08**	**38**	**AT02**	0.0740	0.0000
20	**FR08**	**39**	**HR06**	0.0387	0.0000
20	**FR08**	**40**	**CZ31**	0.0435	0.0000
20	**FR08**	**41**	**RO33**	0.0426	0.0000

Population 1		Population 2		Fst	p value
20	**FR08**	**42**	**RS36**	0.0355	0.0000
20	**FR08**	**43**	**RS37**	0.0309	0.0000
20	**FR08**	**44**	**RS38**	0.0705	0.0000
20	**FR08**	**45**	**RS67**	0.0642	0.0000
20	**FR08**	**46**	**ES41**	0.0857	0.0000
20	**FR08**	**48**	**IT14**	0.0800	0.0000
20	**FR08**	**49**	**IT13**	0.1231	0.0000
20	**FR08**	**51**	**CZ32**	0.0787	0.0000
20	**FR08**	**53**	**IT24**	0.1253	0.0000
20	**FR08**	**54**	**IT26**	0.0967	0.0000
20	**FR08**	**55**	**IT12**	0.1150	0.0000
20	**FR08**	**56**	**RS34**	0.0965	0.0000
20	**FR08**	**57**	**ES40**	0.0784	0.0000
20	**FR08**	**58**	**ES42**	0.0365	0.0000
21	**FR09**	**22**	**FR10**	0.0098	0.0609
21	**FR09**	**23**	**AT01**	0.0399	0.0000
21	**FR09**	**25**	**HR04**	0.0126	0.0200
21	**FR09**	**26**	**HR05**	0.0398	0.0000
21	**FR09**	**27**	**HR07**	0.0318	0.0000
21	**FR09**	**28**	**GR11**	0.0157	0.0240
21	**FR09**	**29**	**CZ30**	0.0132	0.0130
21	**FR09**	**30**	**RS35**	0.0195	0.0020
21	**FR09**	**31**	**IT19**	0.0287	0.0000
21	**FR09**	**32**	**IT20**	0.0474	0.0000
21	**FR09**	**33**	**IT46**	0.0533	0.0000
21	**FR09**	**34**	**IT47**	0.0411	0.0000
21	**FR09**	**35**	**IT17**	0.0682	0.0000
21	**FR09**	**36**	**IT16**	0.0393	0.0000
21	**FR09**	**37**	**ES43**	0.0349	0.0000
21	**FR09**	**38**	**AT02**	0.0766	0.0000
21	**FR09**	**39**	**HR06**	0.0335	0.0000
21	**FR09**	**40**	**CZ31**	0.0366	0.0000
21	**FR09**	**41**	**RO33**	0.0366	0.0000
21	**FR09**	**42**	**RS36**	0.0280	0.0000
21	**FR09**	**43**	**RS37**	0.0235	0.0030
21	**FR09**	**44**	**RS38**	0.0567	0.0000
21	**FR09**	**45**	**RS67**	0.0661	0.0000
21	**FR09**	**46**	**ES41**	0.0891	0.0000
21	**FR09**	**48**	**IT14**	0.0878	0.0000
21	**FR09**	**49**	**IT13**	0.1225	0.0000
21	**FR09**	**51**	**CZ32**	0.0767	0.0000
21	**FR09**	**53**	**IT24**	0.1149	0.0000
21	**FR09**	**54**	**IT26**	0.0933	0.0000
21	**FR09**	**55**	**IT12**	0.0976	0.0000
21	**FR09**	**56**	**RS34**	0.0918	0.0000
21	**FR09**	**57**	**ES40**	0.0802	0.0000
21	**FR09**	**58**	**ES42**	0.0461	0.0000
22	**FR10**	**23**	**AT01**	0.0388	0.0000
22	**FR10**	**25**	**HR04**	0.0156	0.0120
22	**FR10**	**26**	**HR05**	0.0381	0.0000

Population 1		Population 2		Fst	p value
22	**FR10**	**27**	**HR07**	0.0243	0.0030
22	**FR10**	**28**	**GR11**	0.0284	0.0000
22	**FR10**	**29**	**CZ30**	0.0190	0.0050
22	**FR10**	**30**	**RS35**	0.0331	0.0000
22	**FR10**	**31**	**IT19**	0.0499	0.0000
22	**FR10**	**32**	**IT20**	0.0740	0.0000
22	**FR10**	**33**	**IT46**	0.0706	0.0000
22	**FR10**	**34**	**IT47**	0.0548	0.0000
22	**FR10**	**35**	**IT17**	0.0864	0.0000
22	**FR10**	**36**	**IT16**	0.0519	0.0000
22	**FR10**	**37**	**ES43**	0.0598	0.0000
22	**FR10**	**38**	**AT02**	0.1031	0.0000
22	**FR10**	**39**	**HR06**	0.0602	0.0000
22	**FR10**	**40**	**CZ31**	0.0576	0.0000
22	**FR10**	**41**	**RO33**	0.0776	0.0000
22	**FR10**	**42**	**RS36**	0.0630	0.0000
22	**FR10**	**43**	**RS37**	0.0461	0.0000
22	**FR10**	**44**	**RS38**	0.1105	0.0000
22	**FR10**	**45**	**RS67**	0.0895	0.0000
22	**FR10**	**46**	**ES41**	0.1080	0.0000
22	**FR10**	**48**	**IT14**	0.1106	0.0000
22	**FR10**	**49**	**IT13**	0.1533	0.0000
22	**FR10**	**51**	**CZ32**	0.1052	0.0000
22	**FR10**	**53**	**IT24**	0.1339	0.0000
22	**FR10**	**54**	**IT26**	0.1097	0.0000
22	**FR10**	**55**	**IT12**	0.1184	0.0000
22	**FR10**	**56**	**RS34**	0.1097	0.0000
22	**FR10**	**57**	**ES40**	0.1016	0.0000
22	**FR10**	**58**	**ES42**	0.0547	0.0000
23	**AT01**	**25**	**HR04**	0.0324	0.0000
23	**AT01**	**26**	**HR05**	0.0451	0.0000
23	**AT01**	**27**	**HR07**	0.0119	0.0460
23	**AT01**	**28**	**GR11**	0.0167	0.0180
23	**AT01**	**29**	**CZ30**	0.0168	0.0030
23	**AT01**	**30**	**RS35**	0.0330	0.0000
23	**AT01**	**31**	**IT19**	0.0853	0.0000
23	**AT01**	**32**	**IT20**	0.1041	0.0000
23	**AT01**	**33**	**IT46**	0.0961	0.0000
23	**AT01**	**34**	**IT47**	0.0723	0.0000
23	**AT01**	**35**	**IT17**	0.0730	0.0000
23	**AT01**	**36**	**IT16**	0.0389	0.0000
23	**AT01**	**37**	**ES43**	0.0784	0.0000
23	**AT01**	**38**	**AT02**	0.1064	0.0000
23	**AT01**	**39**	**HR06**	0.0685	0.0000
23	**AT01**	**40**	**CZ31**	0.0907	0.0000
23	**AT01**	**41**	**RO33**	0.0803	0.0000
23	**AT01**	**42**	**RS36**	0.0691	0.0000
23	**AT01**	**43**	**RS37**	0.0510	0.0000
23	**AT01**	**44**	**RS38**	0.1003	0.0000
23	**AT01**	**45**	**RS67**	0.1090	0.0000

Population 1		Population 2		Fst	p value
23	**AT01**	**46**	**ES41**	0.1294	0.0000
23	**AT01**	**48**	**IT14**	0.1321	0.0000
23	**AT01**	**49**	**IT13**	0.1632	0.0000
23	**AT01**	**51**	**CZ32**	0.1262	0.0000
23	**AT01**	**53**	**IT24**	0.1383	0.0000
23	**AT01**	**54**	**IT26**	0.1109	0.0000
23	**AT01**	**55**	**IT12**	0.1220	0.0000
23	**AT01**	**56**	**RS34**	0.1198	0.0000
23	**AT01**	**57**	**ES40**	0.0973	0.0000
23	**AT01**	**58**	**ES42**	0.0611	0.0000
25	**HR04**	**26**	**HR05**	0.0232	0.0030
25	**HR04**	**27**	**HR07**	0.0191	0.0060
25	**HR04**	**28**	**GR11**	0.0197	0.0050
25	**HR04**	**29**	**CZ30**	0.0072	0.0889
25	**HR04**	**30**	**RS35**	0.0325	0.0000
25	**HR04**	**31**	**IT19**	0.0365	0.0000
25	**HR04**	**32**	**IT20**	0.0659	0.0000
25	**HR04**	**33**	**IT46**	0.0557	0.0000
25	**HR04**	**34**	**IT47**	0.0452	0.0000
25	**HR04**	**35**	**IT17**	0.0639	0.0000
25	**HR04**	**36**	**IT16**	0.0417	0.0000
25	**HR04**	**37**	**ES43**	0.0477	0.0000
25	**HR04**	**38**	**AT02**	0.0831	0.0000
25	**HR04**	**39**	**HR06**	0.0420	0.0000
25	**HR04**	**40**	**CZ31**	0.0523	0.0000
25	**HR04**	**41**	**RO33**	0.0621	0.0000
25	**HR04**	**42**	**RS36**	0.0427	0.0000
25	**HR04**	**43**	**RS37**	0.0372	0.0000
25	**HR04**	**44**	**RS38**	0.0953	0.0000
25	**HR04**	**45**	**RS67**	0.0861	0.0000
25	**HR04**	**46**	**ES41**	0.1285	0.0000
25	**HR04**	**48**	**IT14**	0.1275	0.0000
25	**HR04**	**49**	**IT13**	0.1661	0.0000
25	**HR04**	**51**	**CZ32**	0.1143	0.0000
25	**HR04**	**53**	**IT24**	0.1258	0.0000
25	**HR04**	**54**	**IT26**	0.1040	0.0000
25	**HR04**	**55**	**IT12**	0.1053	0.0000
25	**HR04**	**56**	**RS34**	0.0964	0.0000
25	**HR04**	**57**	**ES40**	0.1113	0.0000
25	**HR04**	**58**	**ES42**	0.0492	0.0000
26	**HR05**	**27**	**HR07**	0.0215	0.0000
26	**HR05**	**28**	**GR11**	0.0226	0.0080
26	**HR05**	**29**	**CZ30**	0.0250	0.0020
26	**HR05**	**30**	**RS35**	0.0396	0.0000
26	**HR05**	**31**	**IT19**	0.0584	0.0000
26	**HR05**	**32**	**IT20**	0.1009	0.0000
26	**HR05**	**33**	**IT46**	0.0719	0.0000
26	**HR05**	**34**	**IT47**	0.0614	0.0000
26	**HR05**	**35**	**IT17**	0.0809	0.0000
26	**HR05**	**36**	**IT16**	0.0575	0.0000

Population 1		Population 2		Fst	p value
26	**HR05**	**37**	**ES43**	0.0714	0.0000
26	**HR05**	**38**	**AT02**	0.1028	0.0000
26	**HR05**	**39**	**HR06**	0.0582	0.0000
26	**HR05**	**40**	**CZ31**	0.0737	0.0000
26	**HR05**	**41**	**RO33**	0.0648	0.0000
26	**HR05**	**42**	**RS36**	0.0627	0.0000
26	**HR05**	**43**	**RS37**	0.0518	0.0000
26	**HR05**	**44**	**RS38**	0.1150	0.0000
26	**HR05**	**45**	**RS67**	0.0978	0.0000
26	**HR05**	**46**	**ES41**	0.1299	0.0000
26	**HR05**	**48**	**IT14**	0.1178	0.0000
26	**HR05**	**49**	**IT13**	0.1557	0.0000
26	**HR05**	**51**	**CZ32**	0.1155	0.0000
26	**HR05**	**53**	**IT24**	0.1305	0.0000
26	**HR05**	**54**	**IT26**	0.1197	0.0000
26	**HR05**	**55**	**IT12**	0.1268	0.0000
26	**HR05**	**56**	**RS34**	0.1092	0.0000
26	**HR05**	**57**	**ES40**	0.1023	0.0000
26	**HR05**	**58**	**ES42**	0.0651	0.0000
27	**HR07**	**28**	**GR11**	0.0053	0.2068
27	**HR07**	**29**	**CZ30**	0.0089	0.0789
27	**HR07**	**30**	**RS35**	0.0260	0.0000
27	**HR07**	**31**	**IT19**	0.0714	0.0000
27	**HR07**	**32**	**IT20**	0.1086	0.0000
27	**HR07**	**33**	**IT46**	0.0859	0.0000
27	**HR07**	**34**	**IT47**	0.0641	0.0000
27	**HR07**	**35**	**IT17**	0.0776	0.0000
27	**HR07**	**36**	**IT16**	0.0380	0.0000
27	**HR07**	**37**	**ES43**	0.0714	0.0000
27	**HR07**	**38**	**AT02**	0.1107	0.0000
27	**HR07**	**39**	**HR06**	0.0676	0.0000
27	**HR07**	**40**	**CZ31**	0.0899	0.0000
27	**HR07**	**41**	**RO33**	0.0800	0.0000
27	**HR07**	**42**	**RS36**	0.0744	0.0000
27	**HR07**	**43**	**RS37**	0.0435	0.0000
27	**HR07**	**44**	**RS38**	0.1064	0.0000
27	**HR07**	**45**	**RS67**	0.1137	0.0000
27	**HR07**	**46**	**ES41**	0.1372	0.0000
27	**HR07**	**48**	**IT14**	0.1419	0.0000
27	**HR07**	**49**	**IT13**	0.1724	0.0000
27	**HR07**	**51**	**CZ32**	0.1331	0.0000
27	**HR07**	**53**	**IT24**	0.1095	0.0000
27	**HR07**	**54**	**IT26**	0.0984	0.0000
27	**HR07**	**55**	**IT12**	0.0957	0.0000
27	**HR07**	**56**	**RS34**	0.0969	0.0000
27	**HR07**	**57**	**ES40**	0.1058	0.0000
27	**HR07**	**58**	**ES42**	0.0643	0.0000
28	**GR11**	**29**	**CZ30**	0.0063	0.1229
28	**GR11**	**30**	**RS35**	0.0214	0.0060
28	**GR11**	**31**	**IT19**	0.0631	0.0000

Population 1		**Population 2**		**Fst**	**p value**
28	**GR11**	**32**	**IT20**	0.0947	0.0000
28	**GR11**	**33**	**IT46**	0.0740	0.0000
28	**GR11**	**34**	**IT47**	0.0640	0.0000
28	**GR11**	**35**	**IT17**	0.0727	0.0000
28	**GR11**	**36**	**IT16**	0.0351	0.0020
28	**GR11**	**37**	**ES43**	0.0617	0.0000
28	**GR11**	**38**	**AT02**	0.1036	0.0000
28	**GR11**	**39**	**HR06**	0.0542	0.0000
28	**GR11**	**40**	**CZ31**	0.0808	0.0000
28	**GR11**	**41**	**RO33**	0.0596	0.0000
28	**GR11**	**42**	**RS36**	0.0648	0.0000
28	**GR11**	**43**	**RS37**	0.0462	0.0000
28	**GR11**	**44**	**RS38**	0.0819	0.0000
28	**GR11**	**45**	**RS67**	0.0912	0.0000
28	**GR11**	**46**	**ES41**	0.0982	0.0000
28	**GR11**	**48**	**IT14**	0.1127	0.0000
28	**GR11**	**49**	**IT13**	0.1506	0.0000
28	**GR11**	**51**	**CZ32**	0.1049	0.0000
28	**GR11**	**53**	**IT24**	0.0990	0.0000
28	**GR11**	**54**	**IT26**	0.0831	0.0000
28	**GR11**	**55**	**IT12**	0.0870	0.0000
28	**GR11**	**56**	**RS34**	0.0753	0.0000
28	**GR11**	**57**	**ES40**	0.0861	0.0000
28	**GR11**	**58**	**ES42**	0.0574	0.0000
29	**CZ30**	**30**	**RS35**	0.0099	0.0480
29	**CZ30**	**31**	**IT19**	0.0527	0.0000
29	**CZ30**	**32**	**IT20**	0.0854	0.0000
29	**CZ30**	**33**	**IT46**	0.0784	0.0000
29	**CZ30**	**34**	**IT47**	0.0615	0.0000
29	**CZ30**	**35**	**IT17**	0.0564	0.0000
29	**CZ30**	**36**	**IT16**	0.0276	0.0000
29	**CZ30**	**37**	**ES43**	0.0590	0.0000
29	**CZ30**	**38**	**AT02**	0.1060	0.0000
29	**CZ30**	**39**	**HR06**	0.0477	0.0000
29	**CZ30**	**40**	**CZ31**	0.0688	0.0000
29	**CZ30**	**41**	**RO33**	0.0567	0.0000
29	**CZ30**	**42**	**RS36**	0.0521	0.0000
29	**CZ30**	**43**	**RS37**	0.0348	0.0000
29	**CZ30**	**44**	**RS38**	0.0767	0.0000
29	**CZ30**	**45**	**RS67**	0.0978	0.0000
29	**CZ30**	**46**	**ES41**	0.1139	0.0000
29	**CZ30**	**48**	**IT14**	0.1131	0.0000
29	**CZ30**	**49**	**IT13**	0.1581	0.0000
29	**CZ30**	**51**	**CZ32**	0.1027	0.0000
29	**CZ30**	**53**	**IT24**	0.1149	0.0000
29	**CZ30**	**54**	**IT26**	0.0979	0.0000
29	**CZ30**	**55**	**IT12**	0.0858	0.0000
29	**CZ30**	**56**	**RS34**	0.0964	0.0000
29	**CZ30**	**57**	**ES40**	0.0898	0.0000
29	**CZ30**	**58**	**ES42**	0.0601	0.0000

Population 1		**Population 2**		**Fst**	**p value**
30	**RS35**	**31**	**IT19**	0.0493	0.0000
30	**RS35**	**32**	**IT20**	0.0844	0.0000
30	**RS35**	**33**	**IT46**	0.0803	0.0000
30	**RS35**	**34**	**IT47**	0.0618	0.0000
30	**RS35**	**35**	**IT17**	0.0795	0.0000
30	**RS35**	**36**	**IT16**	0.0471	0.0000
30	**RS35**	**37**	**ES43**	0.0657	0.0000
30	**RS35**	**38**	**AT02**	0.1082	0.0000
30	**RS35**	**39**	**HR06**	0.0500	0.0000
30	**RS35**	**40**	**CZ31**	0.0701	0.0000
30	**RS35**	**41**	**RO33**	0.0590	0.0000
30	**RS35**	**42**	**RS36**	0.0543	0.0000
30	**RS35**	**43**	**RS37**	0.0313	0.0000
30	**RS35**	**44**	**RS38**	0.0858	0.0000
30	**RS35**	**45**	**RS67**	0.0826	0.0000
30	**RS35**	**46**	**ES41**	0.1309	0.0000
30	**RS35**	**48**	**IT14**	0.1093	0.0000
30	**RS35**	**49**	**IT13**	0.1598	0.0000
30	**RS35**	**51**	**CZ32**	0.1142	0.0000
30	**RS35**	**53**	**IT24**	0.1089	0.0000
30	**RS35**	**54**	**IT26**	0.1025	0.0000
30	**RS35**	**55**	**IT12**	0.1023	0.0000
30	**RS35**	**56**	**RS34**	0.1106	0.0000
30	**RS35**	**57**	**ES40**	0.1004	0.0000
30	**RS35**	**58**	**ES42**	0.0666	0.0000
31	**IT19**	**32**	**IT20**	0.0128	0.0460
31	**IT19**	**33**	**IT46**	0.0492	0.0000
31	**IT19**	**34**	**IT47**	0.0481	0.0000
31	**IT19**	**35**	**IT17**	0.0896	0.0000
31	**IT19**	**36**	**IT16**	0.0825	0.0000
31	**IT19**	**37**	**ES43**	0.0216	0.0070
31	**IT19**	**38**	**AT02**	0.0687	0.0000
31	**IT19**	**39**	**HR06**	0.0253	0.0020
31	**IT19**	**40**	**CZ31**	0.0238	0.0030
31	**IT19**	**41**	**RO33**	0.0548	0.0000
31	**IT19**	**42**	**RS36**	0.0340	0.0000
31	**IT19**	**43**	**RS37**	0.0431	0.0000
31	**IT19**	**44**	**RS38**	0.0815	0.0000
31	**IT19**	**45**	**RS67**	0.0827	0.0000
31	**IT19**	**46**	**ES41**	0.1396	0.0000
31	**IT19**	**48**	**IT14**	0.1136	0.0000
31	**IT19**	**49**	**IT13**	0.1743	0.0000
31	**IT19**	**51**	**CZ32**	0.1076	0.0000
31	**IT19**	**53**	**IT24**	0.1290	0.0000
31	**IT19**	**54**	**IT26**	0.1166	0.0000
31	**IT19**	**55**	**IT12**	0.1242	0.0000
31	**IT19**	**56**	**RS34**	0.1138	0.0000
31	**IT19**	**57**	**ES40**	0.1165	0.0000
31	**IT19**	**58**	**ES42**	0.0805	0.0000
32	**IT20**	**33**	**IT46**	0.0616	0.0000

Population 1		**Population 2**		**Fst**	**p value**
32	**IT20**	**34**	**IT47**	0.0623	0.0000
32	**IT20**	**35**	**IT17**	0.0898	0.0000
32	**IT20**	**36**	**IT16**	0.0978	0.0000
32	**IT20**	**37**	**ES43**	0.0500	0.0000
32	**IT20**	**38**	**AT02**	0.0681	0.0000
32	**IT20**	**39**	**HR06**	0.0314	0.0000
32	**IT20**	**40**	**CZ31**	0.0277	0.0000
32	**IT20**	**41**	**RO33**	0.0558	0.0000
32	**IT20**	**42**	**RS36**	0.0326	0.0000
32	**IT20**	**43**	**RS37**	0.0400	0.0000
32	**IT20**	**44**	**RS38**	0.0793	0.0000
32	**IT20**	**45**	**RS67**	0.0905	0.0000
32	**IT20**	**46**	**ES41**	0.1386	0.0000
32	**IT20**	**48**	**IT14**	0.1122	0.0000
32	**IT20**	**49**	**IT13**	0.1735	0.0000
32	**IT20**	**51**	**CZ32**	0.1037	0.0000
32	**IT20**	**53**	**IT24**	0.1552	0.0000
32	**IT20**	**54**	**IT26**	0.1408	0.0000
32	**IT20**	**55**	**IT12**	0.1581	0.0000
32	**IT20**	**56**	**RS34**	0.1317	0.0000
32	**IT20**	**57**	**ES40**	0.1218	0.0000
32	**IT20**	**58**	**ES42**	0.0869	0.0000
33	**IT46**	**34**	**IT47**	0.0024	0.3277
33	**IT46**	**35**	**IT17**	0.0873	0.0000
33	**IT46**	**36**	**IT16**	0.0918	0.0000
33	**IT46**	**37**	**ES43**	0.0711	0.0000
33	**IT46**	**38**	**AT02**	0.0829	0.0000
33	**IT46**	**39**	**HR06**	0.0614	0.0000
33	**IT46**	**40**	**CZ31**	0.0524	0.0000
33	**IT46**	**41**	**RO33**	0.0697	0.0000
33	**IT46**	**42**	**RS36**	0.0574	0.0000
33	**IT46**	**43**	**RS37**	0.0593	0.0000
33	**IT46**	**44**	**RS38**	0.1102	0.0000
33	**IT46**	**45**	**RS67**	0.0659	0.0000
33	**IT46**	**46**	**ES41**	0.1684	0.0000
33	**IT46**	**48**	**IT14**	0.1508	0.0000
33	**IT46**	**49**	**IT13**	0.1848	0.0000
33	**IT46**	**51**	**CZ32**	0.1506	0.0000
33	**IT46**	**53**	**IT24**	0.1652	0.0000
33	**IT46**	**54**	**IT26**	0.1537	0.0000
33	**IT46**	**55**	**IT12**	0.1638	0.0000
33	**IT46**	**56**	**RS34**	0.1428	0.0000
33	**IT46**	**57**	**ES40**	0.1417	0.0000
33	**IT46**	**58**	**ES42**	0.0903	0.0000
34	**IT47**	**35**	**IT17**	0.0754	0.0000
34	**IT47**	**36**	**IT16**	0.0695	0.0000
34	**IT47**	**37**	**ES43**	0.0644	0.0000
34	**IT47**	**38**	**AT02**	0.0770	0.0000
34	**IT47**	**39**	**HR06**	0.0537	0.0000
34	**IT47**	**40**	**CZ31**	0.0466	0.0000

Population 1		**Population 2**		**Fst**	**p value**
34	**IT47**	**41**	**RO33**	0.0597	0.0000
34	**IT47**	**42**	**RS36**	0.0497	0.0000
34	**IT47**	**43**	**RS37**	0.0425	0.0000
34	**IT47**	**44**	**RS38**	0.0956	0.0000
34	**IT47**	**45**	**RS67**	0.0663	0.0000
34	**IT47**	**46**	**ES41**	0.1556	0.0000
34	**IT47**	**48**	**IT14**	0.1402	0.0000
34	**IT47**	**49**	**IT13**	0.1727	0.0000
34	**IT47**	**51**	**CZ32**	0.1371	0.0000
34	**IT47**	**53**	**IT24**	0.1397	0.0000
34	**IT47**	**54**	**IT26**	0.1232	0.0000
34	**IT47**	**55**	**IT12**	0.1326	0.0000
34	**IT47**	**56**	**RS34**	0.1234	0.0000
34	**IT47**	**57**	**ES40**	0.1279	0.0000
34	**IT47**	**58**	**ES42**	0.0855	0.0000
35	**IT17**	**36**	**IT16**	0.0237	0.0030
35	**IT17**	**37**	**ES43**	0.0763	0.0000
35	**IT17**	**38**	**AT02**	0.0880	0.0000
35	**IT17**	**39**	**HR06**	0.0346	0.0000
35	**IT17**	**40**	**CZ31**	0.0618	0.0000
35	**IT17**	**41**	**RO33**	0.0343	0.0000
35	**IT17**	**42**	**RS36**	0.0315	0.0000
35	**IT17**	**43**	**RS37**	0.0361	0.0020
35	**IT17**	**44**	**RS38**	0.0573	0.0000
35	**IT17**	**45**	**RS67**	0.0833	0.0000
35	**IT17**	**46**	**ES41**	0.1460	0.0000
35	**IT17**	**48**	**IT14**	0.1228	0.0000
35	**IT17**	**49**	**IT13**	0.1774	0.0000
35	**IT17**	**51**	**CZ32**	0.1368	0.0000
35	**IT17**	**53**	**IT24**	0.1603	0.0000
35	**IT17**	**54**	**IT26**	0.1436	0.0000
35	**IT17**	**55**	**IT12**	0.1499	0.0000
35	**IT17**	**56**	**RS34**	0.1401	0.0000
35	**IT17**	**57**	**ES40**	0.1206	0.0000
35	**IT17**	**58**	**ES42**	0.0887	0.0000
36	**IT16**	**37**	**ES43**	0.0748	0.0000
36	**IT16**	**38**	**AT02**	0.1038	0.0000
36	**IT16**	**39**	**HR06**	0.0516	0.0000
36	**IT16**	**40**	**CZ31**	0.0677	0.0000
36	**IT16**	**41**	**RO33**	0.0497	0.0000
36	**IT16**	**42**	**RS36**	0.0465	0.0000
36	**IT16**	**43**	**RS37**	0.0403	0.0000
36	**IT16**	**44**	**RS38**	0.0683	0.0000
36	**IT16**	**45**	**RS67**	0.0802	0.0000
36	**IT16**	**46**	**ES41**	0.1154	0.0000
36	**IT16**	**48**	**IT14**	0.1186	0.0000
36	**IT16**	**49**	**IT13**	0.1514	0.0000
36	**IT16**	**51**	**CZ32**	0.1147	0.0000
36	**IT16**	**53**	**IT24**	0.1383	0.0000
36	**IT16**	**54**	**IT26**	0.1150	0.0000

Population 1		**Population 2**		**Fst**	**p value**
36	**IT16**	**55**	**IT12**	0.1252	0.0000
36	**IT16**	**56**	**RS34**	0.1221	0.0000
36	**IT16**	**57**	**ES40**	0.0975	0.0000
36	**IT16**	**58**	**ES42**	0.0476	0.0000
37	**ES43**	**38**	**AT02**	0.0560	0.0000
37	**ES43**	**39**	**HR06**	0.0215	0.0070
37	**ES43**	**40**	**CZ31**	0.0350	0.0000
37	**ES43**	**41**	**RO33**	0.0566	0.0000
37	**ES43**	**42**	**RS36**	0.0393	0.0000
37	**ES43**	**43**	**RS37**	0.0595	0.0000
37	**ES43**	**44**	**RS38**	0.0788	0.0000
37	**ES43**	**45**	**RS67**	0.1041	0.0000
37	**ES43**	**46**	**ES41**	0.1062	0.0000
37	**ES43**	**48**	**IT14**	0.0916	0.0000
37	**ES43**	**49**	**IT13**	0.1552	0.0000
37	**ES43**	**51**	**CZ32**	0.0984	0.0000
37	**ES43**	**53**	**IT24**	0.1433	0.0000
37	**ES43**	**54**	**IT26**	0.1165	0.0000
37	**ES43**	**55**	**IT12**	0.1269	0.0000
37	**ES43**	**56**	**RS34**	0.1193	0.0000
37	**ES43**	**57**	**ES40**	0.1140	0.0000
37	**ES43**	**58**	**ES42**	0.0844	0.0000
38	**AT02**	**39**	**HR06**	0.0369	0.0000
38	**AT02**	**40**	**CZ31**	0.0391	0.0000
38	**AT02**	**41**	**RO33**	0.0609	0.0000
38	**AT02**	**42**	**RS36**	0.0390	0.0000
38	**AT02**	**43**	**RS37**	0.0796	0.0000
38	**AT02**	**44**	**RS38**	0.0888	0.0000
38	**AT02**	**45**	**RS67**	0.0790	0.0000
38	**AT02**	**46**	**ES41**	0.1489	0.0000
38	**AT02**	**48**	**IT14**	0.1282	0.0000
38	**AT02**	**49**	**IT13**	0.1850	0.0000
38	**AT02**	**51**	**CZ32**	0.1229	0.0000
38	**AT02**	**53**	**IT24**	0.2013	0.0000
38	**AT02**	**54**	**IT26**	0.1814	0.0000
38	**AT02**	**55**	**IT12**	0.2000	0.0000
38	**AT02**	**56**	**RS34**	0.1663	0.0000
38	**AT02**	**57**	**ES40**	0.1255	0.0000
38	**AT02**	**58**	**ES42**	0.0898	0.0000
39	**HR06**	**40**	**CZ31**	0.0153	0.0060
39	**HR06**	**41**	**RO33**	0.0107	0.0350
39	**HR06**	**42**	**RS36**	0.0030	0.2667
39	**HR06**	**43**	**RS37**	0.0146	0.0190
39	**HR06**	**44**	**RS38**	0.0337	0.0000
39	**HR06**	**45**	**RS67**	0.0575	0.0000
39	**HR06**	**46**	**ES41**	0.1069	0.0000
39	**HR06**	**48**	**IT14**	0.0711	0.0000
39	**HR06**	**49**	**IT13**	0.1300	0.0000
39	**HR06**	**51**	**CZ32**	0.0732	0.0000
39	**HR06**	**53**	**IT24**	0.1289	0.0000

Population 1		**Population 2**		**Fst**	**p value**
39	**HR06**	**54**	**IT26**	0.1165	0.0000
39	**HR06**	**55**	**IT12**	0.1275	0.0000
39	**HR06**	**56**	**RS34**	0.1147	0.0000
39	**HR06**	**57**	**ES40**	0.0809	0.0000
39	**HR06**	**58**	**ES42**	0.0610	0.0000
40	**CZ31**	**41**	**RO33**	0.0406	0.0000
40	**CZ31**	**42**	**RS36**	0.0126	0.0200
40	**CZ31**	**43**	**RS37**	0.0436	0.0000
40	**CZ31**	**44**	**RS38**	0.0731	0.0000
40	**CZ31**	**45**	**RS67**	0.0729	0.0000
40	**CZ31**	**46**	**ES41**	0.1333	0.0000
40	**CZ31**	**48**	**IT14**	0.1036	0.0000
40	**CZ31**	**49**	**IT13**	0.1749	0.0000
40	**CZ31**	**51**	**CZ32**	0.0962	0.0000
40	**CZ31**	**53**	**IT24**	0.1524	0.0000
40	**CZ31**	**54**	**IT26**	0.1354	0.0000
40	**CZ31**	**55**	**IT12**	0.1543	0.0000
40	**CZ31**	**56**	**RS34**	0.1319	0.0000
40	**CZ31**	**57**	**ES40**	0.1138	0.0000
40	**CZ31**	**58**	**ES42**	0.0770	0.0000
41	**RO33**	**42**	**RS36**	0.0043	0.2138
41	**RO33**	**43**	**RS37**	0.0176	0.0050
41	**RO33**	**44**	**RS38**	0.0120	0.0350
41	**RO33**	**45**	**RS67**	0.0428	0.0000
41	**RO33**	**46**	**ES41**	0.1059	0.0000
41	**RO33**	**48**	**IT14**	0.0808	0.0000
41	**RO33**	**49**	**IT13**	0.1255	0.0000
41	**RO33**	**51**	**CZ32**	0.0759	0.0000
41	**RO33**	**53**	**IT24**	0.1193	0.0000
41	**RO33**	**54**	**IT26**	0.1018	0.0000
41	**RO33**	**55**	**IT12**	0.1179	0.0000
41	**RO33**	**56**	**RS34**	0.1045	0.0000
41	**RO33**	**57**	**ES40**	0.0892	0.0000
41	**RO33**	**58**	**ES42**	0.0817	0.0000
42	**RS36**	**43**	**RS37**	0.0179	0.0050
42	**RS36**	**44**	**RS38**	0.0311	0.0000
42	**RS36**	**45**	**RS67**	0.0473	0.0000
42	**RS36**	**46**	**ES41**	0.1246	0.0000
42	**RS36**	**48**	**IT14**	0.0860	0.0000
42	**RS36**	**49**	**IT13**	0.1393	0.0000
42	**RS36**	**51**	**CZ32**	0.0837	0.0000
42	**RS36**	**53**	**IT24**	0.1383	0.0000
42	**RS36**	**54**	**IT26**	0.1261	0.0000
42	**RS36**	**55**	**IT12**	0.1366	0.0000
42	**RS36**	**56**	**RS34**	0.1175	0.0000
42	**RS36**	**57**	**ES40**	0.1021	0.0000
42	**RS36**	**58**	**ES42**	0.0758	0.0000
43	**RS37**	**44**	**RS38**	0.0383	0.0000
43	**RS37**	**45**	**RS67**	0.0677	0.0000
43	**RS37**	**46**	**ES41**	0.1173	0.0000

Population 1		**Population 2**		**Fst**	**p value**
43	**RS37**	**48**	**IT14**	0.0920	0.0000
43	**RS37**	**49**	**IT13**	0.1361	0.0000
43	**RS37**	**51**	**CZ32**	0.0922	0.0000
43	**RS37**	**53**	**IT24**	0.1045	0.0000
43	**RS37**	**54**	**IT26**	0.0939	0.0000
43	**RS37**	**55**	**IT12**	0.1002	0.0000
43	**RS37**	**56**	**RS34**	0.0948	0.0000
43	**RS37**	**57**	**ES40**	0.0836	0.0000
43	**RS37**	**58**	**ES42**	0.0594	0.0000
44	**RS38**	**45**	**RS67**	0.0798	0.0000
44	**RS38**	**46**	**ES41**	0.1269	0.0000
44	**RS38**	**48**	**IT14**	0.0996	0.0000
44	**RS38**	**49**	**IT13**	0.1397	0.0000
44	**RS38**	**51**	**CZ32**	0.0927	0.0000
44	**RS38**	**53**	**IT24**	0.1470	0.0000
44	**RS38**	**54**	**IT26**	0.1244	0.0000
44	**RS38**	**55**	**IT12**	0.1355	0.0000
44	**RS38**	**56**	**RS34**	0.1285	0.0000
44	**RS38**	**57**	**ES40**	0.1255	0.0000
44	**RS38**	**58**	**ES42**	0.1053	0.0000
45	**RS67**	**46**	**ES41**	0.1820	0.0000
45	**RS67**	**48**	**IT14**	0.1418	0.0000
45	**RS67**	**49**	**IT13**	0.1811	0.0000
45	**RS67**	**51**	**CZ32**	0.1512	0.0000
45	**RS67**	**53**	**IT24**	0.1833	0.0000
45	**RS67**	**54**	**IT26**	0.1699	0.0000
45	**RS67**	**55**	**IT12**	0.1954	0.0000
45	**RS67**	**56**	**RS34**	0.1668	0.0000
45	**RS67**	**57**	**ES40**	0.1379	0.0000
45	**RS67**	**58**	**ES42**	0.0913	0.0000
46	**ES41**	**48**	**IT14**	0.0491	0.0000
46	**ES41**	**49**	**IT13**	0.1255	0.0000
46	**ES41**	**51**	**CZ32**	0.0447	0.0000
46	**ES41**	**53**	**IT24**	0.2132	0.0000
46	**ES41**	**54**	**IT26**	0.1703	0.0000
46	**ES41**	**55**	**IT12**	0.1933	0.0000
46	**ES41**	**56**	**RS34**	0.1696	0.0000
46	**ES41**	**57**	**ES40**	0.1075	0.0000
46	**ES41**	**58**	**ES42**	0.1240	0.0000
48	**IT14**	**49**	**IT13**	0.0808	0.0000
48	**IT14**	**51**	**CZ32**	0.0147	0.0110
48	**IT14**	**53**	**IT24**	0.2139	0.0000
48	**IT14**	**54**	**IT26**	0.1855	0.0000
48	**IT14**	**55**	**IT12**	0.2095	0.0000
48	**IT14**	**56**	**RS34**	0.1908	0.0000
48	**IT14**	**57**	**ES40**	0.0829	0.0000
48	**IT14**	**58**	**ES42**	0.1067	0.0000
49	**IT13**	**51**	**CZ32**	0.1063	0.0000
49	**IT13**	**53**	**IT24**	0.2193	0.0000
49	**IT13**	**54**	**IT26**	0.1912	0.0000
49	**IT13**	**55**	**IT12**	0.2176	0.0000
49	**IT13**	**56**	**RS34**	0.1966	0.0000
49	**IT13**	**57**	**ES40**	0.0969	0.0000
49	**IT13**	**58**	**ES42**	0.1564	0.0000
51	**CZ32**	**53**	**IT24**	0.2026	0.0000
51	**CZ32**	**54**	**IT26**	0.1722	0.0000
51	**CZ32**	**55**	**IT12**	0.1928	0.0000
51	**CZ32**	**56**	**RS34**	0.1762	0.0000
51	**CZ32**	**57**	**ES40**	0.0940	0.0000
51	**CZ32**	**58**	**ES42**	0.1100	0.0000
53	**IT24**	**54**	**IT26**	0.0124	0.0659
53	**IT24**	**55**	**IT12**	0.0172	0.0190
53	**IT24**	**56**	**RS34**	0.0253	0.0060
53	**IT24**	**57**	**ES40**	0.1741	0.0000
53	**IT24**	**58**	**ES42**	0.1855	0.0000
54	**IT26**	**55**	**IT12**	0.0141	0.0539
54	**IT26**	**56**	**RS34**	0.0253	0.0030
54	**IT26**	**57**	**ES40**	0.1637	0.0000
54	**IT26**	**58**	**ES42**	0.1600	0.0000
55	**IT12**	**56**	**RS34**	0.0227	0.0050
55	**IT12**	**57**	**ES40**	0.1783	0.0000
55	**IT12**	**58**	**ES42**	0.1866	0.0000
56	**RS34**	**57**	**ES40**	0.1598	0.0000
56	**RS34**	**58**	**ES42**	0.1620	0.0000
57	**ES40**	**58**	**ES42**	0.0789	0.0000

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