Biosystematics and Ecological Approaches to the Study of *Q. pubescens* s.l. in Southern Italy

Antonio Luca Conte

Department of Bioscience and Territory

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Antonio Luca Conte

Matricola 157311

Tutor

Prof. Paola Fortini

Co-tutor

Prof. Romeo Di Pietro

Coordinator

Prof. G. Fabbrocino

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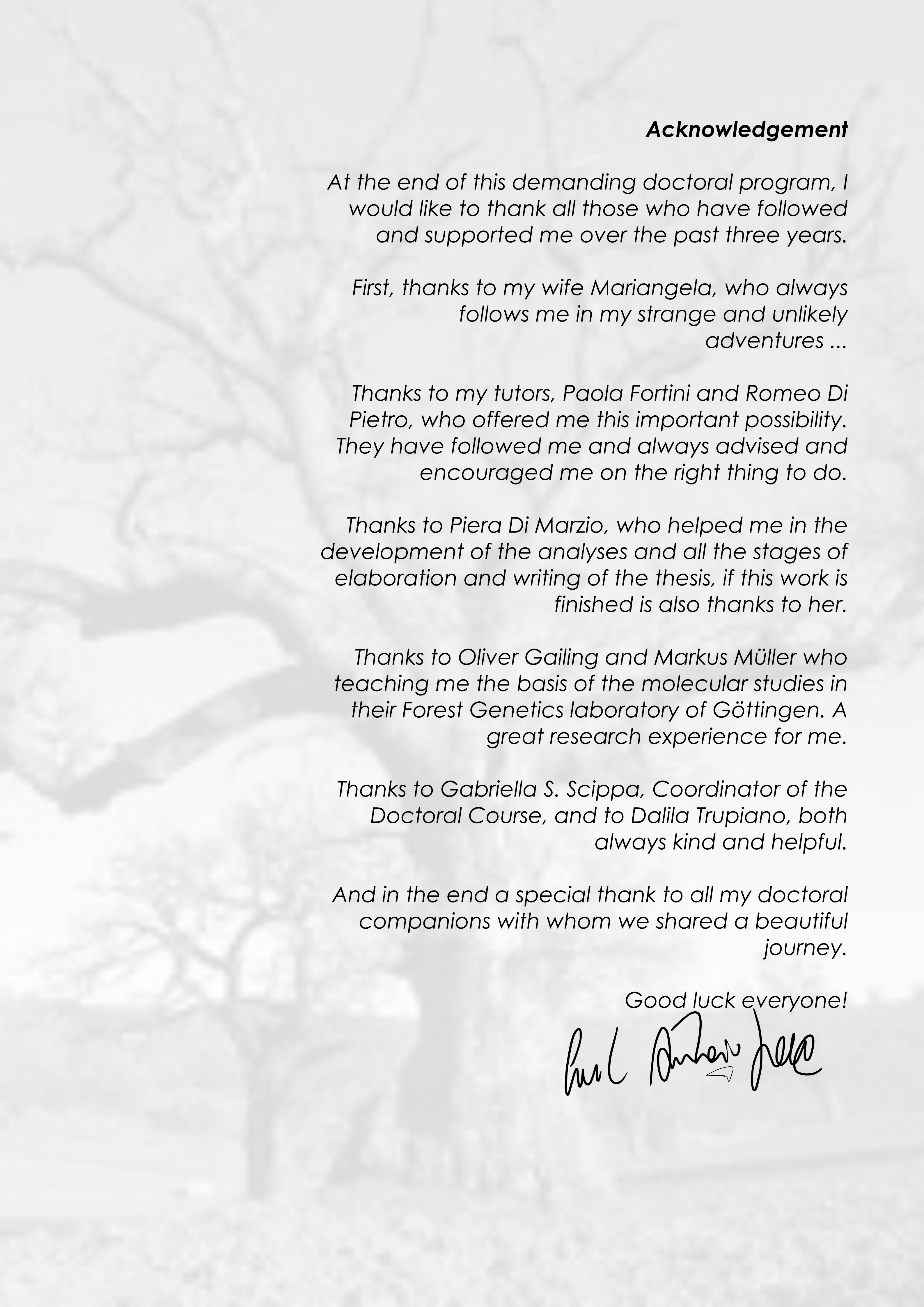

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Good luck everyone!

‘Downy Oak’ Appennino Lucano Val d'Agri Lagonegrese National Park (Basilicata, Italy).

Contents

Preface

To date, over 60.000 species of trees and shrubs cover of about 1/3 of our global land area (FAO, 2015; Keenan et al., 2015; Beech et al., 2017). The largest forest habitats and highest phanerophytic diversity are located within the inter-tropical region of the Neotropical and Paleotropical floral kingdoms (sensu Good, 1947; and Takhtajan, 1986)[1], with over 23,000 tree species. However, a significant part of the global distribution of the forest habitats can also be found in the forest biomes occurring in the Holarctic floral kingdoms, where almost 6,000 species of trees have currently been identified (Beech et al., 2017). Europe occupies the north-western area of the Holarctic kingdom, and a considerable area of its surface - 33% or 215 million ha - is covered by forests (De Rigo et al., 2016). A great part of these European forest communities, especially in southern Europe, is characterised by the dominance of species belonging to the genus *Quercus* L. This genus exhibits a mainly boreal distribution that, in Europe, is expressed by 38 taxa at species rank (Euro+Med Plant Base, 2014) that become 78 if subspecies and hybrid forms are included (Govaerts et al., 2019)[2]. The most recent figures at the global level report for over 435 species of oaks throughout the world (Kremer et al., 2019).

The genus *Quercus* L.[3] is the most numerous group in the family of *Fagaceae* (Subfamily *Quercoideae*). The first remain of this genus appeared on this planet around 56 Ma (Hipp et al., 2019), and its species vastly expanded in the Eocene and Oligocene epochs (Denk et al., 2017; Hipp et al., 2019). Its current distribution area includes Central America, North America (mainly along the East coast) Eurasia up to its easternmost limit (Cina, Indochina, Korean Peninsula and Japan). The highest species richness is found in Mexico and Eastern Asia. According to Cavender-Bares (2016, 2019), oaks represent the most important contributors in providing forest biomass of the whole Americas.

Oaks are longevous trees, that show a very variable structure. Some of them are majestic trees (e.g. *Quercus robur, Q. petraea*), generally 20 to 30 meters high (about 40 m maximum), while some others have a shrubby structure (e.g. *Q. coccifera, Q. calliprinos*) and are typical of shrubland habitats such as the Mediterranean maquis. All the oaks have reproductive organs on different branches, being monoecious species. They can use multiple reproduction strategies such as seeding, sapling or vegetative propagation. The spirally arranged leaves have deciduous and inconspicuous stipules, and there are two main types of habitus, evergreens or deciduous. The lamina can be malacophylla or sclerophyll with petiole or without sessile, lobed or non-lumbar. The margins can be different for species: whole, toothed or toothed with extensions similar to bristles. The inflorescences are unisexual and arranged on axils of leaves or flakes of buds, generally grouped at the base of a new bud (Pignatti et al., 1982, 2017-2019; Pasta et al., 2016). Oaks are a diploid species that show a stable ploidy, bearing 2n=24 chromosomes, x=12 (Aykut et al., 2008; Peruzzi et al., 2016; Asgharpour et al., 2019),

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[1] According to Cox (2001) and Cox et al. (2016), the names and distribution of some floral kingdoms must be reconsidered.

[2] Many authors have tried to elaborate an exhaustive checklist of the species belonging to the genus *Quercus*. According to Beech et. al. (2017), the number of oak's entities in the world is 424, 29 in European countries. While according to Trehane (2007), the list of all accepted names of oaks in the world, is composed of 1,600 entities (including hybrids and subspecies), 114 are belonging to European taxa.

[3] Often in this work of thesis, I used the common name 'oaks' to refer to the taxonomic group of *Quercus* L.

with a few episodic triploid individuals mentioned in the literature (Butorina, 1993; Naujoks et al., 1995; Dzialuk et al., 2007). The number of chromosomes 2n=24 (x=12) appears stable as well within the *Fagaceae* family (Mehra et al., 1972; Ohri & Ahuja 1990, 1991; D'Emerico et al., 1995).

Oaks are well adapted to several habitats, especially in the tropical zone. Many species have colonised arid and semi-arid areas in the open forest of Afghanistan, Spain, Italy, North Africa, Central America and the Middle East. Others have colonised the humid forests and the riparian habitats in the wetlands of the Southeast coast of the USA, or the riparian forests of the Danube River. We can also find species of oaks in the subalpine forests of the Alborz Mountains in northern Iran (De Rigo et al., 2016; Denk et al., 2017; Kremer et al. 2012, 2019).

In the recent 2015 United Nations Climate Change Conference (COP 21), one of the most relevant arguments was the conservation of forests (Poorter et al., 2015). The role of the forest in the mitigation of climate change is only one of the essential ecosystem services in which the oak forest is involved. Forests also play an important role in the regulation of other ecosystem services such as hydro-geology services, water cycle, the reservoir of biodiversity, habitat building and socio-economic activities (e.g. carpentry, furniture, cabinet making, veneer, cask industry, fuelwood, hunting and fungus- and berry-gathering services) (De Rigo et al., 2016).

Oaks have been investigated, especially in the recent past, regarding their genetic structure and forest tree adaptation in order to understand their molecular mechanisms or to study climate change's effect on the forest habitat (Durand et al., 2010; Gentilesca et al., 2017; Peñuelas et al., 2017; Turco et al., 2018; Conte et al., 2019). The Quercus species are fundamental in the structure of forest ecosystems and play a primary role in the conservation of many other species which develop in their undergrowth (Kremer & Hipp, 2019). In addition, the oaks seem to play an important role also in the ecological research applied at the study of zoonotic-diseases. Recently has been discovered their role as the predictors of risk of the zoonotic outbreak in the Lyme-disease in the USA (Ostfeld et al., 2006; Krawczyk et al., 2020).

Oaks has also deeply influenced human history. The acorn of the oak has represented abundant food, and the remains of the consumption of acorns have been found in Israel (dating of 800,000 years ago; Goren-Inbar et al., 2002), as well as in Central and North America, Asia and Europe (Fagan, 2004; Anderson, 2007; Denk et al., 2007). Oaks have provided wood for millennia (e.g. De'Athe et al., 2013; Out, 2017; Ruiz-Alonso et al., 2017), used to warm, to produce utensils and furniture and much more. Many religions and cultures are linked with some species of this group, demonstrating a strong cultural and economic relationship with mankind.

In this Doctor of Philosophy thesis (PhD), I have chosen to investigate some species of the European 'white oaks' (*sensu* Denk et al., 2017)[4] with a biosystematic approach. Biosystematic studies allow obtaining data and information through a wide range of disciplines such as genetics, morphology, ecology and some other more.

My PhD's thesis aims to clarify the biosystematic relationships and taxonomical status among some critic taxa of European white oaks in southern Italy. This group, more commonly called pubescent oaks (*sensu* Di Pietro et. al., 2018) is composed of *Quercus amplifolia* Guss., *Q. apennina* Lam., *Q. congesta* C.Presl., *Q. dalechampii* Ten., *Q. humilis* DC, *Q. ichnusae* Mossa, Bacch. & Brullo,

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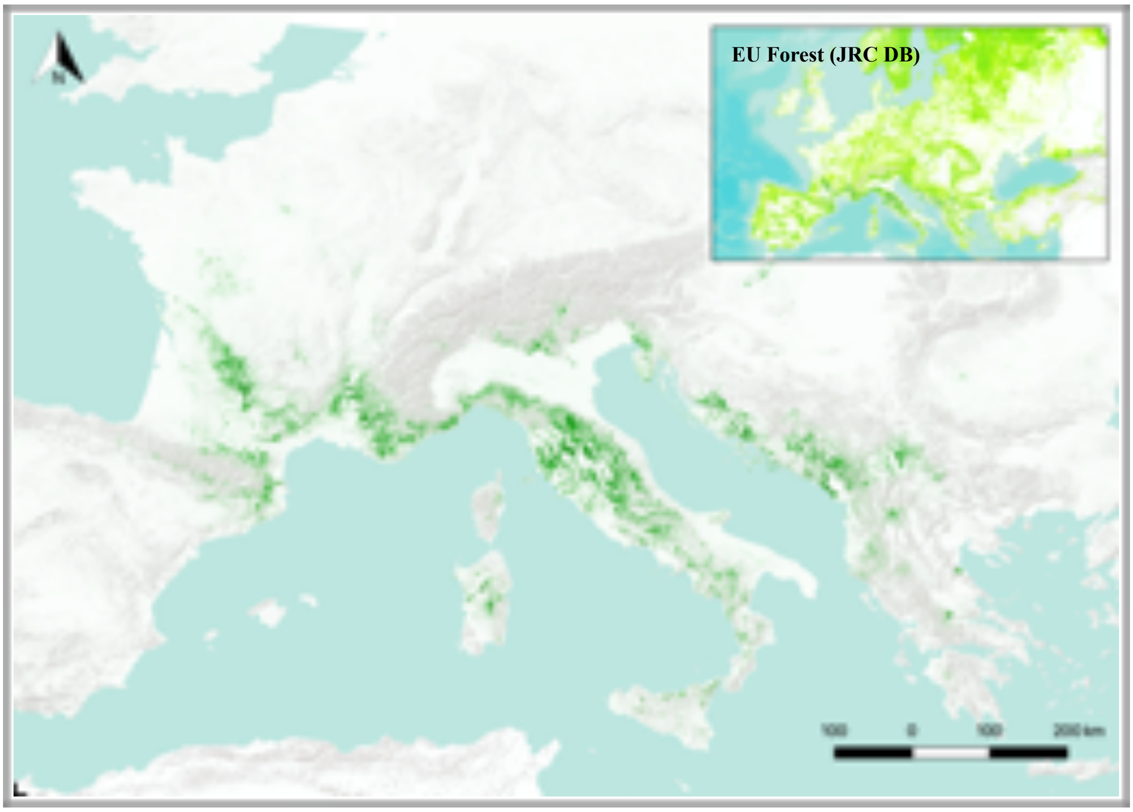
[4] 'White oaks' is a common name used to describe a wide group of American and European oaks that are included in the genus *Quercus*, Sect. *Quercus* (*sensu* Denk et al. 2017) (see also Schwarz' classification, genus *Quercus*, Subgenus *Lepidobalanus*, 1937).

Q. leptobalana Guss.[5], *Q. virgiliana* (Ten.) Ten. Some of these taxa are considered as ”doubtful” species since they exhibit a large overlapping of morphological and ecological characters and are oftentimes found in sympatry. For these reasons, these species are frequently attributed to the group of *Q. pubescens* s.l. (sensu lato) (Map. 1.0).



Map 1.0: Distribution map of *Quercus pubescens* s.l. in the Mediterranean basin, elaborated by forest cover map of the EC Joint Research Centre (Kempeneers et al. 2011).

I have divided my PhD research work into three different sections. In the first part of the thesis, an in-depth study about the origin and paleogeographic history of oaks in the last 60 Ma[6], and an overview of the biosystematic issues of the Italian pubescent oaks were presented. In chapter one, *‘Paleogeographic and Evolutionary History of White Oaks’*, I have rebuilt the path of evolution and distribution of this group, especially through Europe and the Mediterranean basin.

In chapter two, I focused on the *‘Biosystematics of White Oaks’*. Starting from the first attempts to describe and classify these oaks, I have reconstructed their taxonomic vicissitudes and nomenclatural history in southern Europe and, more specifically, in Italy.

The second part of the thesis is composed by two chapters (formatted as scientific articles submitted), chapter three ‘*A multivariate morphometric analysis of phytognostic traits in southern Italy and Sicily pubescent-oaks’* and chapter four ‘*Does the genetic diversity among pubescent white oaks in southern Italy, Sicily and Sardinia Islands support the current taxonomic classification?’.*

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[5] This taxon is cited in different ways in the different Floras and check-lists, e.g *Quercus leptobalanus* Guss. (Govaerts et al., 2019), *Quercus leptobalana* Guss. (Bartolucci et al., 2018), *Quercus leptobalanos* Guss. (Brullo et al. 1999). In this work, I have adopted the binomial *Quercus leptobalana* Guss. according to Bartolucci et al. (2018).

[6] Ma, means mega annum. A unit of time equal to one million, or 10^6 years. The suffix ‘Ma’ is commonly used in scientific disciplines such as geology, palaeontology to signify very long time periods into the past or future (Wikipedia, 2019).

This part of the thesis deal with the differences that emerge among oak individuals and populations collected in Southern Italy on the basis of morphological and molecular investigations. According to recent publications (Viscosi et al. 2009, 2012; Fortini et al., 2015a,b), the use of a combinate morphometric and molecular approach allows providing a more consistent taxonomic assignment to be compared to how is proposed in the original diagnosis (protologues) of the oak taxa names. For this reason, I have collected samples (leaves and acorns of tree individuals) in oak populations located precisely in the proximity of the *'loci classici'* of the aforementioned oak taxa which occurred in the Calabria, Sicilia and Sardinia regions. A total of 396 tree individuals was collected.

In chapter five (composed by a published article), '*Oak decline in the Mediterranean basin: a study case from the southern Apennines*', I studied the effects that regional episodes of climate change may have on the oak forests habitat, threatening their conservation.

The last part of the thesis, chapter six '*Synthesis and outlook',* is a summary of the main results obtained and a look at future implications of this research.

At the end of this work, in chapter seven, '*Paper contributions*', I have introduced some papers to which I have contributed.

Chapter 1. Paleogeographic and Evolutionary History of the White Oaks

1.1 The first Cenozoic remains in Northern Hemisphere

According to Barrón et al. (2017), remains of the presence of the genus *Quercus* L. (*Quercoideae, Fagaceae*) in the Northern Hemisphere appear in the fossil record from the Palaeogene (66-23 Ma) to the Neogene (23-2.5 Ma). The fossils have been recovered from many localities in North America and Europe in the Late Cretaceous, and Palaeogene floras show many similarities with the leaf feature included in a concept group of *Quercus*. The floras that come from the Oligocene (33-23 Ma) and Quaternary in the Northern Hemisphere contain many leaves, fruit fossils and pollens, attributable with certainty to the genus *Quercus* L. However, some of these remains require a re-examination with new information and approaches. The oldest mesofossil that shows affinity with a primordial ancestral parent of *Quercus* and its family of *Fagaceae* is *Archaefagacea futabensis* gen. et sp. nov., from the Early Coniacian period in Japan (Takahashi et al., 2008a). Pieces of evidence of the presence of the Cretaceous fagalean fossils coming from pollen record a so-called Normapolles complex. This complex is one of the essential fossil records for the Angiosperm coming from the Late Cretaceous. A significant amount of palynofloras from the Late Cretaceous period in the Northern Hemisphere are included in the Normapolles complex (Friis et al., 2011). Their maximum geographic distribution extends from eastern North America and Europe to western Siberia in a continuous floristic realm, referred to as the Normapolles Province (Batten, 1981; Herngreen & Chlonova, 1981; Zaklinskaya, 1981; Herngreen et al., 1996). The earliest fossil evidence of the genus *Quercus* comes from North America, in the Cenomanian Dakota Formation of Kansas (Lesquereux, 1892) and the Black Hills, South Dakota (Ward, 1899). A similar kind of fossil has been described by Fontaine (1889) from Late Cretaceous floras of North America and Europe (e.g. Dorf, 1942; Němejc & Kvaček, 1975; Váchová & Kvaček, 2009). Another kind of form similar to the *Quercus* was included in the *Quercophyllum* group (Fontaine, 1889).

The North American strata have provided a considerable number of fossils, based on the descriptions of the leaves and fruits coming from the Palaeogene of Wyoming, North Dakota, Montana and Colorado (Brown, 1962; Budantsev & Golovneva, 2009), although, according to Manchester (2014), they probably do not all belong to forms related to *Quercus*.

The first Asian *Quercus* fossils, *Quercus cretaceoxylon*, come from the Upper Cretaceous and were found on Hokkaido Island, Japan (Suzuki & Ohba, 1991). The earliest continental Asian *Quercus* fossils were found in the Russian Far east, *Q. tsagajanica Pojark* (Iljinskaja, 1982). Most Cretaceous records coming from Asia are poorly preserved fossils, which prevents a specific identification of the group to which they belong, especially for the forms assignable to the *Quercus* shape (Jones, 1986; Zhou, 1993; Xing et al., 2013; Barrón et al., 2017).

In Europe (western Eurasia), possibly due to the particularly complex European palaeogeographic history in the Cretaceous and Palaeogene, no one European Cretaceous fossil remains are attributable to the genus *Quercus*. Clear trace of this genera is present in Europe from the Palaeocene-Eocene transition (55 Ma) in France (Vincent et al., 1977; Michon & Merle, 2001) and at Sant Pankras, Austria (Hofmann et al., 2011). The palynoflora of Sant Pankras (Austria) (ibid.[7]) testifies to a particular climatic condition in this area with a 'warm temperate evergreens-deciduous forest'. Probably based on the local geomorphology conditions of the territory, this kind of subtropical flora exhibited some temperate elements, because the climatic features were warm and wet, but not strictly tropical. The pollen of this Austrian site shares some similarity with the recent group of *Ilex*, but this evidence, according to Denk et al. (2012), is not assigned to this group.

The biogeographical history of the genus *Quercus* has been much influenced by the geological and tectonic issue of the last 80 Ma, especially in the Mediterranean Basin. The disappearance of Tethys[8], the Alpine-Himalayan orogenesis and the movements of plates in southern Europe have played a principal role. At the same time, the relationships between these endogenous and exogenous factors, and climatic changes, have influenced the repeated process of vegetation colonising and then disappearing from large areas of Eurasia. The vegetation of the Northern Hemisphere reached a distribution similar to the present day only at the end of the last glaciation, 11,000 BP[9] (Dryas). Between 8,000 and 6,000 BP, the European flora appeared similar to its present-day distribution, thanks to the stabilisation of the climate and the geological movements. Reconstructing the history of this important group of the forest ecosystem has been carried out by starting from the fossil records of leaves, pollen and also wood, fruits and flowers. The purpose of this work is to collect bibliographical information from the most recent research in the field of palaeobotany in order to create an up-to-date review of the knowledge of the origins of the genus *Quercus* and its distribution in the southern Europe.

1.2 A brief history of the Mediterranean Basin (from Mesozoic to Cenozoic, 145 Ma to present)

The evolution and distributions of the morphotypes belonging to the genus *Quercus* in the present European continent were influenced especially by the geological and consequent ecological modifications which have characterised the Mediterranean Basin starting from the Cenozoic climatic oscillations (Medail et al., 2017).

In the Mesozoic Era, the area occupied by the current Mediterranean Basin was the site of a Mesozoic ocean, called Tethys. This ocean was located between the continents of Gondwana and Laurasia, disappearing during the Cretaceous Period with the opening of the Indian and Atlantic Oceans (Carballo et al., 2015). In the Middle Cenozoic (Miocene), the Tethys Ocean was drastically

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[7] Ibid., adverb, means in the same source (used to save space in textual references to a quoted work which has been mentioned in a previous reference).

[8] Tethys Ocean, a Mesozoic ocean located between the continents of Gondwana and Laurasia (200 Ma).

[9] BP, means Before Present years. It is a time scale used in archaeology and geology and other scientific disciplines to specify when events occurred in the past. The standard practice is to use 1 January 1950 as the commencement date (present epoch) of the age scale that refers to the origin of the radiocarbon dating in the 1950s. This abbreviation is also interpreted as "Before Physics". Because the nuclear weapons testing artificially altered the proportion of the carbon isotopes in the atmosphere, making dating after that time likely to be unreliable (Wikipedia, 2019).

reduced. The western end of the Tethys became part of the current Mediterranean Sea. The geographical aspect of the Mediterranean Basin between the Oligocene and the Miocene was not so far to that we observe at nowadays, although it was composed of a complex set of large and small internal basins (Thompson, 2005).

At the end of the Mesozoic and throughout the whole Cenozoic Era geological forces, led mainly by the Alpine-Himalayan (Cretaceous-Tertiary) orogenesis, provoked the transformation of the edges of both the Eurasia subcontinent and the Mediterranean Basin (Popov et al., 2006). Already in the Miocene, the paleo-Mediterranean Basin was bordered by low thresholds and numerous mountain ranges - the Atlas, the Sierra Nevada, the Pyrenees, the Apennines, Dinarids, Taurus, and the Anatolia plateau. Between the Pliocene and the Pleistocene, especially, there was a powerful development in height of these mountainous ranges with a consequent fragmentation.

During the Cenozoic, the distribution of animals and plants was strongly influenced by the migration routes of the Mediterranean's microplates (e.g. Adria-Iberian and Cyrno-Sardinian microplates). The Iberian microplate has undergone several shifts due to the Alpine-Himalayan orogeny and the movement of the European plate. From the Late Jurassic to the Late Cretaceous it suffered the thrust that led it to clash with Europe, creating the Pyrenees chain. The Adriatic microplate was an offshoot of the African continent located in the area currently occupied by the Adriatic Sea and was probably composed of a more or less continuous sequence of islands. This plate partially connected southern Europe with Africa. In the Permian, the opening of the Ionian Sea led to the separation of the Adria microplate from Africa and its movement towards the area currently occupied by the Apulian region in southern Italy. In the Late Oligocene (35-30 Ma), Corsica and Sardinia were part of a unique continent between the block of Eurasia and Africa, located north-west of their current position and connected with the Hercynian massifs (Hsü, 1971; Westphal et al., 1976; Cohen, 1980; Cherchi & Montadert, 1982). North-eastern Corsica, Calabria, the Kabyles (in North Africa) and the Betic Cordillera were linked to one another in an Alpine Belt that extended around the southern edge of the Hercynian massifs (Alvarez, 1976), a mountain system dated back to the Carboniferous. The Cyrno-Sardinian microplate began to rotate south-eastwards (Alvarez, 1974; Rosenbaum et al., 2002), and this movement caused the fragmentation and consequent migration of some Tyrrhenian islands and Calabrian Arc (Cherchi & Montadert, 1982; Robertson & Grasso, 1995) and the onset of the opening of the Balearic Basin. Corsica achieved its current position after collision with the crust of the Northern Apennines (~20 Ma). The Sardinia Island and the Calabrian Arc continued to rotate south-eastern wards until their collision with the Tunisian margin of North Africa at ~14 Ma (Alvarez, 1974). Consequently, in the Middle Miocene, Sardinia became separated from the Calabrian Arc. In the meanwhile, the aperture of the Tyrrhenian Sea occurred (about 9-5 Ma). Corsica, Sardinia and the other fragments of the initial microplate separated from the Balearic Islands and southern France since the Miocene. The Balearic Islands, from their part, had repeated connections among each other (Minorca and Majorca had their latest connection in the Pleistocene) and with the Iberian Peninsula (Thompson, 2005).

1.3 The Messinian salinity crisis

Since the beginning of the Phanerozoic (541 Ma), global climate conditions have undergone an evolution, with gradual trends from warming to cooling (Mills et al., 2018). However, the most recent climate changes in the last two eras (Mesozoic and Cenozoic) have been the most severe for plants and animals globally. In the last 65 Ma, the climate changes have been driven by tectonic processes, by rhythmic and periodic cycles induced by orbital phenomena, and rarely, by rapid aberrant shifts and extreme climate transients due to a mix of factors (Zachos et al., 2001). In the Cenozoic era, one of the most relevant geological events that caused a great climatic upheaval in the Mediterranean area was the Messinian salinity crisis.

The Messinian salinity crisis began in 5.96 Ma (Krijgsman et al., 1999) and involved the whole Mediterranean Basin for around 600,000 years. This event was due to the tectonic movements in the Late Miocene (~8 Ma), which caused the closing of the strait between the Atlantic Ocean and the Mediterranean. The level of the Mediterranean Sea dropped due to the evaporation of the water and the climate became drier. This condition caused the formation of a mosaic of large lakes in which extensive evaporites were deposited. In these lake deposits, researchers have found some cyanobacteria and other fossils typical of shallow water (Hsü et al., 1973). So they have demonstrated that this significant accumulation of the salt deposits occurred in many cycles of desiccation-inundation, which probably repeated ~8-10 times during the Messinian. During the Messinian salt crisis, large areas of the southern shores of the Mediterranean Basin probably looked like a desert (Hsü, 1973; Faquette et al., 2006). Geologists have recognised three main stages of the evolution of the Messinian crisis. In the first stage, the evaporation of water created some shallow sub-basins; in the second stage, where the crisis reached its peak, the evaporite precipitation shifted to the deepest depocentres; and in the third stage, the Mediterranean Basin was characterised by large-scale environmental fluctuations and it looked like a brackish water lake (Roveri et al., 2014; Medail et al., 2017). The climatic zone of the Mediterranean and the Black Sea Basin before the Messinian crisis was characterised by a warm and humid climate, except for some small areas like Spain, North Africa and Sicily, which were warm and dry. In the first phase of the Messinian crisis, the Mediterranean climate showed the same climatic conditions as before the crisis. The pollen records show high regional variability, however, the climatic condition in the southern Mediterranean region was probably characterised by a drier environment that stayed more or less stable before, during and after the crisis (Faquette et al., 2006). These extraordinary changes in habitat were a dramatic episode for some species and an extraordinary opportunity for others. The Messinian crisis influenced the migration of plants and animals, creating land-bridge connections among such places as Corsica, Sardinia and the north of Italy; there were connections from Sicily to southern Italy and North Africa and, by different terrestrial bridges in the Aegean Sea, from continental Greece to the south-east across the Aegean Islands and to Crete via the Peloponnese. In the passage from the Miocene to the Pliocene, the opening of the Strait of Gibraltar and the establishment of a permanent connection between the Atlantic and the Mediterranean put an end, suddenly, to the salt crisis of the Messinian at 5.33 Ma (Krijgsman et al., 1999). Since then, the coastlines of the Mediterranean Basin have maintained locations similar to the current positions. However, in the Quaternary, the oscillations due to the cycles of glaciations caused many variations in the sea level. In the Last Glacial Maximum, the coastlines were ~150m lower than the present sea level (Kaiser, 1969). A minor effect on the European vegetation was caused by local or regional volcanism events (Rosenbaum & Lister, 2004) in the Calabrian Arc, around the Vesuvius area, in Sicily (Etna), in the Greek Islands, in North Africa and Turkey.

1.4 Quaternary Ice Age (2.58 to the present)

In the last period of the Cenozoic era, the geological events had a minor influence on the distribution of vegetation, because a minor number of events occurred. In the Quaternary age, southern Europe achieved a sort of geological stability which was interrupted only by local volcanos activities and isolated earthquakes. On the contrary, the climatic oscillations had greater importance in determining the distribution of plant species and vegetation both in the boreal zones and Mediterranean areas. The most important events that characterised the Quaternary period were the extensive cold seasons (glaciations) which characterised Earth's middle and high latitudes. At the global scale, the glaciations occurred with different modalities and intensities according to the region where they occurred as demonstrated by the asynchronicity of glacial maxima across the world during the last glacial cycle (i.e. the Weichselian, Wurmian, Wisconsinan Stage, MIS 5d-Early 1) (Ehlers et al., 2011) (Figure 1.1).

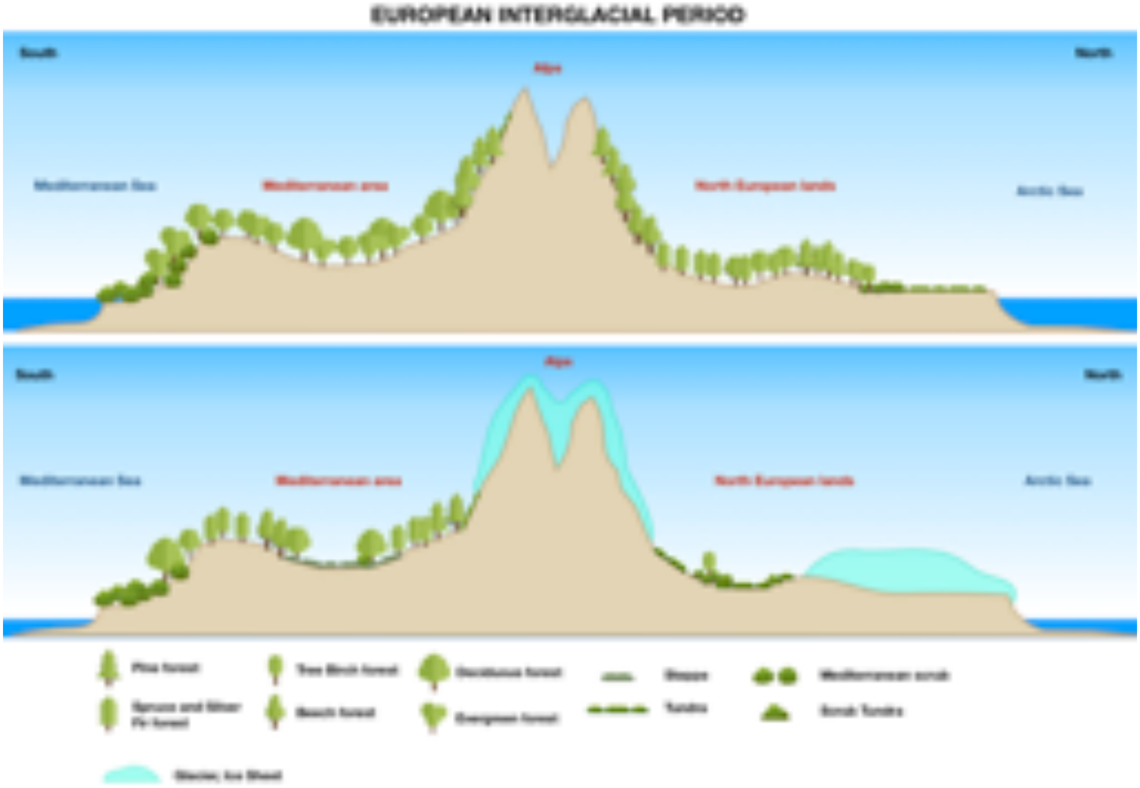

Fig. 1.1 Schemes of the vegetation distribution during interglacial and glacial cycles in the Quaternary (redesigned for this thesis, inspired by Woodward, 2014).

The Last Glacial Maximum started receding around 20,000 BP, and the new cold event, the so-called Younger Dryas (12,900-11,700 BP), temporarily reversed the gradual climatic warming (Meltzer et al., 2014). The decreasing of the temperature from the North (at the southern limits of permafrost) to the South was probably of the order of 5-16°C (Kaiser, 1969). A marked seasonality of rainfall occurred (Thompson, 2005), and the typical Mediterranean plants persisted through this period in isolated glacial refugia, mainly in the Iberian Peninsula, Italy and Greece (Petit et al., 2003). From the end of the Pleistocene to the Holocene, the Earth's climate system has been involved in a significant transformation linked to sudden changes in atmospheric and oceanic circulation that have brought a global increase of the temperatures. However, at the end of Pleistocene, there was a return to cold conditions (Ehlers et al., 2011). In the recent Younger Dryas (12,900-11,700 BP), forests retreated again in Europe and steppe vegetation spread (Pons & Reille, 1988; Woodward, 2014; Bertini, 2015), and *Betula* pollen showed a marked decline in some sites (Turner & Hannon, 1988). In the last period of climatic oscillations, that is, 18,000-10,000 BP, *Prunus* pollen became more abundant in some regions, along with the pollen of other bush species, as did *Juniperus* and *Cistus* (Thompson, 2005). The abundance of these species thus appeared to predominate in the landscape, suggesting a moderately cold climate. Around 10,000 BP, the stabilisation of the Mediterranean climate began, with more definitive warming. From west to east, deciduous oak forests with *Corylus*, *Alnus*, *Fraxinus*, *Betula*, *Ulmus*, and *Tilia,* covered large areas on the slopes of the Mediterranean mountains (Pons et al., 1995; Grove & Rackham, 2001; Bertini, 2003, Denk et al., 2019). Forest occurrence was less abundant in the eastern and southern parts of Mediterranean than in the north-west part of the basin. In southern Spain, the return of forest vegetation was rapid, probably thanks to the numerous refugia of glacial maxima. Since the Last Glacial Maximum, the distributions of the forest, maquis and steppe vegetation in Europe were in line with the influence of the Mediterranean climate. The major modifications that have subsequently occurred have significantly been due to human activities (Thompson, 2005).

1.5 Recolonisation after the last glacial period in Europe (~0.0100 Ma)

Many habitats in southern Europe have been used as refugia by the Mediterranean vegetation during the glacial ages. The concept of 'refugia' however, have been sometimes used in a misleading way (Feliner, 2011). According to Médail and Diadema (2009), a refugium is: *'an area where distinct genetic lineages have persisted through a series of Tertiary or Quaternary climate fluctuations owing to special, buffering environmental characteristics'*. Feliner (2011) stated that *'rather than erasing species or populations, the adverse climatic conditions caused their movement across a wide possibility of routes and altitudes in relation with the regional topography'*. This implied that differentiation in allopatry, achieved in a variety of niches was sometimes interrupted, or at least influenced by new contacts. These events have generated gene flow, hybridisation and even hybrid speciation at the homoploid or polyploid level (ibid.). Thus, the main processes that occurred in the southern European peninsulas across the ice ages were probably related with preservation, accumulation, complete or incomplete differentiation, and an admixture of species in the spatially stable areas (hybrid zones) (Feliner, 2011). But the history and the locations of the refugia in Europe could be more complex. According to Magri et al. (2007, 2010), there are more pieces of evidence about the persistence of some wood species (*Abies*, *Corylus* and *Quercus*) in central Europe and the Mediterranean Basin during the ice ages. In these locations, the local climatic condition did not have to be much different from that of the present day (cf. Medail & Diadema, 2009).

The most recent isopollen maps of *Quercus* (oak) describe the pollen percentages across Europe for 12,000, 10,000, 8,000, 6,000, 4,000 and 2,000 BP, with radiocarbon dating (De Rigo et al., 2016). The interpretation of this map provides a widescreen view of the distributions of the *Quercus* after the Last Glacial Maximum period. Starting from the main refugia located in southern Europe (Iberia, Italy and Greece) the recolonisations of oaks toward central Europe were fairly rapid until the end of the Last Glacial Maximum. When the Mediterranean and European climate became stable, the radiation of the distribution of *Quercus* was very fast, so that 6,000 years ago it had regained all of Europe except for the Scandinavia peninsula. The evidence-based on the pollen record, according to Brewer et al. (2002), suggests that we can recognise two steps in the spread of *Quercus* in the European landscapes. First, in the late-glacial interstadial (13-11,000 BP), *Quercus* spread towards the central European mountains from the southern refugia of Iberia, Italy and Balkans. Second, after the stabilisation of the climate, to one more favourable to deciduous trees species, the oak spread rapidly into north-west Europe. Due to geographical barriers and ecological competitions, the spread into central and eastern Europe was slower during the Holocene. The information provided from the other historical footprints (pollen deposits) and by genetic fingerprints [chloroplast DNA (cpDNA) polymorphisms] (Petit et al., 1997; Brewer et al., 2002; Kremer, 2002; Petit et al., 2002a, b) has added some additional data to this story. The sharing of the same cpDNA haplotype by different white oak species occupying the same stands indicates that hybridisation was extensive during postglacial recolonisation (Petit et al., 1997). Brewer et al. (2002) suggested that the average speed of the migration from refugia was extremely rapid, calculating around 300 to 500 metres per year (Brewer et al., 2002). Besides, Petit (2002) proved that despite the strong founder effects that accompanied the recolonisation, oaks were able to maintain their genetic diversity. The highest diversity has been found in southern Europe, in the alleged refugia (Petit et al., 2002a; Medail & Diadema 2009). Petit et al. (2003) also hypothesised that the fast recolonisation stages towards central Europe were possible precisely for events of interspecific hybridisation and the consequent increase of genetic diversity. These two factors have improved the capacity of oak in colonising the new habitats available.

1.6 The origins of the genus *Quercus*

Findings of fossil oaks from the same epoch in two continents mean that it is almost impossible at present to exactly establish the place of origin of oaks (Baskin & Baskin, 2016). Oaks spread northwards, towards higher latitudes already in the Palaeocene (66-56 Ma). Subsequently, during the Eocene (56-33.9 Ma), the palaeoclimatic and palaeogeographic conditions became more favourable for oaks' survival and development so that these conditions facilitated a rapid spread over the whole Holarctic region (Baskin & Baskin, 2016; Simeone et al., 2016).

Both molecular and fossil evidence support the idea of a wide presence of the *Quercus* genus at the end of the Eocene (c.35 Ma) when all the significant lineages of oaks were already present from high to mid-latitudes (Denk et al., 2012; Bouchal et al., 2014; Hubert et al., 2014; Grímsson et al. 2015, 2016; Barrón et al., 2017). Some recent studies suggested that the split of many forms of *Fagaceae* occurred already in previous periods (from the Cretaceous to the Palaeogene), whereas the significant evolution of all the sections of the genus *Quercus* seems to have occurred from 55 to 35 Ma (Hofmann, 2010; Friis et al., 2011; Hofmann et al., 2011; Manchester, 2011; Hubert et al., 2014; Simeone et al., 2016; Hipp et al., 2019).

In this broad framework of data based on fossil records, palaeogeographic reconstructions and molecular evidence, three hypotheses are currently considered as the most probable among palaeobotanists.

The first hypothesis (Zhou, 1992) assumes that oaks originated in south-eastern Asia, from a parental group of the genus *Trigonobalanus* during the end of the Tertiary (66-56 Ma). Hereafter, a global colonisation phase began, and led the oaks to colonise the most of the Northern Hemisphere, moving westwards, from Europe to North America, using the North Atlantic land bridge and Bering Strait (Jeong et al., 2009; In Sung Paik et al., 2012).

The second hypothesis (Manos & Stanford, 2001) reports as a centre of origin of *Quercus* genus a large area of the Boreal Hemisphere occupied by a deciduous tropical-boreal forest. The colonisation in the two continents, Asia and North America, occurred because of allopatric speciation of two related taxa from a common ancestor. From these two centres of origin, oaks spread rapidly in the Oligocene and Miocene in response to crucial global climate changes and using land bridges occurring around the North Pole for trans-continental migrations

The third hypothesis, expressed variously by many authors, establishes that, between Palaeocene and Eocene, the genus *Quercus*, as well as several other tree taxa, took advantage from possible land bridge connections (Grímsson, 2008; Rui-Qi Li et al., 2013; Zhang et al., 2013; Baskin & Baskin, 2016; Simeone et al., 2016). This meant that the origin of oaks could have occurred in some areas located at high latitudes in the Northern Hemisphere. From those places of origin, oaks, together with a floristic component related to oak woods, would have been migrated from the western side of North Atlantic towards Europe. This latter hypothesis would be supported by many fossils dating back to the Paleocene found at high latitudes (North America, Greenland, Northern Europe) (Crepet & Nixon, 1989; Manchester, 1994; Grímsson et al., 2015) and mid-latitudes (Central Europe, South East Asia) (Kvaček & Walther, 1989; Hofmann, 2010).

A similar point of view is presented by Simeone et al. (2016), who yet suggested that oaks originated in the sub-Arctic regions of the Northern Hemisphere and subsequently split into two groups. The Arctic lineage became the *'New World Clade'* whereas a second lineage, *'Old World Clade'* migrates in the subtropical areas and gave rise to a geographic and taxonomic fragmentation (Simeone et al., 2016). The hypothesis that *'the elements of Eurasia and North America are coming from a unique area at the high-latitude'* (Simeone et al., 2016) has been supported in the past by many authors (see Baskin & Baskin, 2016). Engler (1882), argues that the Cenozoic plant assemblages of the Arctic region are similar to the recent Northern temperate woody flora, which he called the *'Arcto-Tertiary Element'*. Meanwhile, he called the southern equivalent the *'Palaeo-tropical Element',* which comprise families today distributed in the Old World Tropics. Chaney (1959) called *'Arcto-Tertiary-Geoflora'* the *'Arcto-Tertiary Element'* (in the sense of Engler). Many other studies demonstrate the presence of the same floristic groups across North America, Europe, and eastern Asia (Gray, 1840, 1846, 1859, 1878; Fernald, 1931; Graham, 1972; Wood, 1972; Boufford & Spongberg, 1983; Wu, 1983; Boufford, 1992; Hong, 1993; Li, 1952, 1972; Manchester, 1999; Qian, 1999-2002; Wen, 1999; Guo & Ricklefs, 2000; Xiang et al., 1998-2000). Regarding this similarity of the Paleo-Tertiary flora, Wolfe and Tiffney proposed the Boreotropical hypothesis (Wolfe, 1975-1977; Tiffney, 1985 a, b). This hypothesis was based on the occurrence of tropical climatic conditions at the northern latitudes during the Eocene. This common condition at the same latitudes, which have allowed the development and the exchange of tropical taxa via land bridges between the *'New'* and the *'Old World'* tropical flora.

According to Baskin & Baskin (2016), in the Eocene forests, tropical evergreens, temperate deciduous, boreal and alpine taxa could be found as the result of multiple waves of migration whose specific composition of migrants was related to the different types of climatic conditions (Tiffney, 1985a; Xiang et al., 1998; Wen, 1999; Donoghue & Smith, 2004; Pennington & Dick, 2004).

Almost all the hypotheses origin and spread of the genus *Quercus* highlight on the important role played by the northern land bridges (Davis et al., 2002-2004; Weeks et al., 2005; Zerega et al., 2005; Muellner et al., 2006; Smedmark & Anderberg, 2007; Merckx et al., 2008; Erkens et al., 2009). However, according to other authors, the *'long-distance dispersal by water and wind currents, rather than by tectonic history/vicariance would be the most likely explanation for many transcontinental taxa'* (Winkworth et al., 2002; Queiroz, 2005; Crisp et al., 2009; de Li et al., 2011; Baskin & Baskin, 2016).

A significant update about the timing and direction of the split of *Quercus* genus during the Cenozoic comes from the recent work of Hipp et al. (2019), about '*the Genomic landscape of the global oak phylogeny*'. These authors have re-calculated the timing of the global oak phylogeny (cf. Hubert et al., 2014) stating that by the Mid-Eocene (45 Ma), all Sections of genus *Quercus* (in the sense of Denk et al., 2017) had already originated, except for the Section *Quercus*, which origin they fixed at the Eocene-Oligocene boundary (33 Ma).

Hipp et al. (2019) describe a situation whereby, in the Eocene, despite the cooling of the climate, the genus *Quercus* survived in the northern temperate-boreal zone, whereas in the same period, other oak species continued to inhabit the southern subtropical zones (sections *Virentes*, *Lobatae*, and *Quercus;* Cavender-Bares, 2019). In the rest of the Northern Hemisphere, the subtropical and evergreens broadleaf forests of Eurasia (Deng et al., 2018) were dominated by Section *Cyclobalanopsis*, whereas some species of Sections *Cerris* and *Ilex* were widespread in the Mediterranean basin (Hipp et al., 2019).

1.7 Final considerations

In the last 20 years, a large amount of fossil evidence and molecular data have been reported in several research papers. Ancestors of the European species of oaks occurred at the high latitude between North America and North Europe before the Paleogene. According to some pieces of evidence, the first parental form of oaks may have originated in some of these areas of Northern Hemisphere.

Despite some interesting hypotheses, however, there is not enough evidence to firmly establish that the place of origins of *Quercus* genus was located in the Arctic zones of the Northern Hemisphere.

In the Upper Cretaceous, the northlands of the Boreal Hemisphere were linked by land bridges between the western area of North America and the eastern area of Europe (North Atlantic land bridge) and also between the eastern area of Asia and the western area of North America (Bering land bridge). Accordingly, the migration and distribution of the oaks started between the Cretaceous and the Cenozoic and continued during the Neogene.

The distribution of the species of *Quercus* in Eurasia and North America was probably driven by the climate oscillations of the Cenozoic, during which we observed a long cooling period intermixed with shorter significantly warmer periods which allowed the Paleotropical tertiary element to penetrate in the arctic regions in the north of both Eurasia and North-America.

In the Upper-Lower Miocene (Messinian), the *Quercus* species had a great expansion. However, with the Messinian crisis, this group was forced to reduce its range due to the severe climatic conditions which took place in the Mediterranean Basin. The palaeogeographic history of oaks in the last 5.33 Ma (after the Messinian crisis) was strongly related to the climatic oscillations of the Plio-Pleistocene period that caused a continuous replacement of habitat and floras. The climatic and palaeogeographic conditions became more stable only at the end of the Dryas Ice Age (11,700 BP).

The first remains of genus *Quercus* in the North Atlantic land, come from Axel Heiberg Island Eocene strata. The first European remains of the Sect. *Quercus* come from the central area of the continent and date back to the Oligocene. Moreover, in some strata of Germany and Kazakhstan probable forms of the ancestors of white oaks emerged, of which *Q. furuhjelmii* is the most interesting. Another potential ancestor of white oaks is *Q. hispanica*, from the Late Miocene of Spain. This latter is a species similar to existing *Q. humilis* Mill. (in most of the floras synonymised to *Q. pubescens* Willd.) and *Q. faginea* Lam. spp. *faginea* (Barrón et al., 2014). In the Middle Miocene, *Q. hispanica* was an important element of the humid and summer-wet forest in Europe, together with *Quercus drymeja* and *Q. mediterranea*. Spanning the entire Neogene, the remains of oaks are common in many strata of Eurasia and North America, which is a sign that the distribution of this group in this chronostratigraphic period has been very important (Figure 1.2).

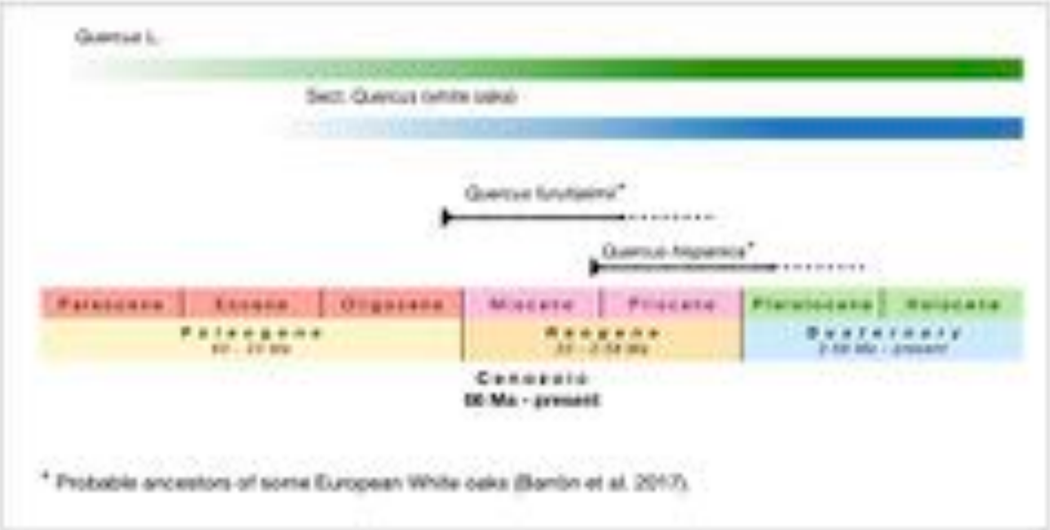

Fig. 1.2 Chronogram of the first remains of the genus *Quercus* L., sect. *Quercus*.

At the end of Miocene with the Messinian crisis, a period of climatic instability began, and forest communities dominated by deciduous species would be replaced by a steppe community dominated by *Artemisia* genus.

The recent Quaternary history of *Quercus* in Europe is characterised by a great southern migration, due to the cycling cooling of the climate that produced a great coverage of ice in the North and middle Europe. The main refuges for the temperate and sclerophyllous trees were along the coast, in the lower valleys, in the small southern mountain chains and hills in the Iberian Peninsula, Italy, Greece and the Middle East.

From the Mediterranean Basin at the end of the glaciation, a first migration started flowing towards the North. The latter episode was stopped by a minor climatic crisis, called the Dryas (11,000 BP). However, the migration restarted after the Dryas period and many oaks' species reached all parts of Europe, except the Scandinavian peninsula, over 8,000 years. Between 6,000-4,000 BP the surface of Europe was already covered by a vegetation community very similar to the present day. But around 12,000 BP, the human activity had already started to changes the face of North Africa and Europe.

Chapter 2. Biosystematics of the White Oaks

2.1 The taxonomy of the genus *Quercus* L. in an international context

The genus *Quercus* (oaks) has had a complex taxonomic history in which many authors participated describing new species, proposing new names, substituting old ones, and, sometimes, contributing in creating a confusing taxonomic situation in the long run. Recent studies have clarified some taxonomic aspects (Kremer et al., 2012; Cavender-Bares et al., 2015; Denk et al., 2017; Kremer & Hipp, 2019; Hipp et al., 2019), helping to return to a more taxonomically consistent classification framework. In this chapter, I have followed the exhaustive taxonomical framework proposed by Denk et al. (2017).

Carl Linnaeus was the first author to describe oaks species. In *Species Plantarum* (1753, Vol 2), Linnaeus described fourteen species of oaks, from North America and Europe, which were referred to the following main groups: white oaks, red oaks, cerris oaks and ilex oaks (*sensu* Denk et al., 2017). Regarding the European white oaks, he described only *Q. æsculus* [= *Q. petraea* (Matt.) Liebl.] and *Q. robur* (Table 2.1).

Another author who contributed to the knowledge of the genus *Quercus* in Europe was Carl Ludwig Willdenow. This author published several works from 1787 to 1816, in which he described twenty species, four of which belonging to the European white oaks (Table 2.1). Among the species described by Willdenow there was the quite common but still undefined, *Quercus pubescens* Willd. 1805.

In the first half of the nineteenth century, John Loudon who interested in a global approach to the study of the oaks described 150 species. In two reviews published in 1838 and 1839, he provided the first infra-generic classification of genus *Quercus* and established the fundamental subdivision of European oaks in Sections (sections *Cerris, Ilex,* and *Robur*; Table 2.1), which corresponds to the three major clades resulting from the recent molecular-phylogenetic trees published in Denk et al. (2017).

In 1871, Anders Ørsted studied a new group of Asian oaks (*Lithocarpus*: the cycle-cup oaks) and proposed a new classification where he assigned the Asian group to the genus *Cyclobalanopsis* (subtribe *Quercinae*) keeping it separated from the genus *Quercus* (Table 2.1). Ørsted's concept was subsequently followed by researchers such as Camus (1936-1938) and Nixon (1993) and it is currently in use in the *Flora of China* (Huang et al.,1999; Flora of China, 2016).

Between 1878 and 1986, a large number of other notable authors have studied oaks (for instance, Nyman, Boissier, Borzì, Lojacono, De Candolle, Ascherson & Graebner, Reichinger, Krüssmann, Trelease). But in the early twentieth century, two authors - Camus and Schwarz - became the most important contributors to the knowledge of the taxonomy of oaks.

Camus and Schwarz were influenced by Ørsted's global concept, but they proposed two different visions, mainly for the grouping of North American and Eurasian white oaks (Table 2.1). Camus (1934, 1936) divided the genus *Quercus* into two subgenera, *Cyclobalanopsis* Schneid. and *Euquercus* Nickel & Camus. Schwarz (1936) proposed a new classification based on a tribe system composed of

Cyclobalanopsideae and *Querceae*. In these tribes, Schwarz recognised four genera: *Cyclobalanopsis*, *Erythrobalanus, Macrobalanus,* and *Quercus*.

Table 2.1 Taxonomic scheme of the genus *Quercus*, referring only to the classification of the European White Oaks.

Author	Genus	Subgenus	Sections	Sub-Sections	Species
Linneus (1753)	*Quercus*				*Q. æsculus* (= *Q. petraea* (Matt.) Liebl.), *Q. robur*
Willdenow (1796, 1805)	*Quercus*				*Q. austriaca, Q. bicolor, Q. canariensis, Q. discolor, Q. elongata, Q. elongata, Q. maritima, Q. montana, Q. mucronata, Q. myrtifolia, Q. nana, Q. prinoides, Q. pubescens, Q. pyrenaica, Q. rigida, Q. sericea, Q. tinctoria, Q. tomentosa, Q. tournefortii, Q. turneri*
Loudon (1838-1839)	*Quercus*	*Cerris*			*Q. cerris* L., *Q. macrolepis* Kotschy, *Q. lanuginosa* Lam.= *Q. cerris* L.
		Ilex			*Q. coccifera* L., *Q. ilex* L., *Q. suber* L.
		Robur			*Q. esculus* L.=*Q. petraea*, *Q. pyrenaica* Willd., *Q. pedunculata* Ehrh=*Q. robur* L. *Q. apennina* Lam.=*Q. pubescens* L., *Q. sessiliflora* Sal.=*Q. petraea*
Ørsted (1871)	*Quercus*	*Cerris*	*Eucerris*		*Q. cerris* L., *Q. lanuginosa* Lam.=*Q. cerris*
		Lepidobalanus	*Eulepidobalanus*		*Q. frainetto* Ten., *Q. pubescens* Willd., *Q. conferta* Ait.=*Q. frainetto*, *Q. haas* Kotschy=*Q. robur*, *Q. pedunculata* Ehrh=*Q. robur*, *Q. sessiliflora* Sal.=*Q. petraea*, *Q. toza* Bosc.=*Q. pyrenaica*
Camus (1936)	*Quercus*	*Euquercus*	*Cerris*	*Eucerris*	*Q. cerris* L.
			Lepidobalanus	*Sessiliflorae*	*Q. Sessilis* Ehrh., *Q. lanuginosa* Lam. (included: subsp. *eu-lanuginosa* var. *leptobalanus* Lojac., var. *congesta* Presl, var. *gussonei* Borzì var. *cupaniana* Guss., var. *virgiliana* Ten., var. *amplifolia* Borzì subsp. *dalechampii* Camus subsp. *medwedirwii* Camus subsp. *crispata* Camus subsp. *brachyphylla* Camus subsp. *palensis* Camus)
				Pedunculatae	*Q. robur* Lin., *Q. brutia* Ten. *Q. borzii* Camus
			Mesobalanus	*Macrantherae*	*Q. frainetto* ten., *Q. toza* Bosc.=*Q. pyrenaica*
		Cerris	*Eucerris*		*Q. cerris* L., *Q. lanuginosa* Lam.=*Q. cerris* L.

Table 2.1 Taxonomic scheme of the genus *Quercus*, referring only to the classification of the European White Oaks.

Author	Genus	Subgenus	Sections	Sub-Sections	Species
Schwarz (1937)	*Quercus*	*Quercus*	*Dascia*	*Esculus*	*Q. congesta* Presl., *Q. frainetto* Ten., *Q. pubescens* Willd., *Q. pyrenaica* Willd., *Q. sicula* Borzì, *Q. brachyphilla* Kotschy=*Q. pubescens, Q. esculiformis* O. Schwarz=*Q. frainetto, Q. virgiliana* Ten.=*Q. pubescens*
			Robur		*Q. robur* L., *Q. robur* subsp. *brutia*
			Roburoides	*Roburiformis*	*Q. dalechampii* Ten., *Q. petraea* (Matt.) Liebl.
Menitsky (1984)	*Quercus*	*Cerris*		*Cerris*	*Q. cerris* L., *Q. lamuginosa* Lam.=*Q. cerris*
		Quercus		*Galliferea*	*Q. pubescens* Willd.
				Macranterae	*Q. frainetto* Ten.
				Quercus	*Q. petraea* Matt (liebl.), *Q. robur* L., *Q. pedunculiflora* K.Koch=*Q. robur* L.
Denk et al. (2017)	*Quercus*	*Quercus*	*Quercus*		*Q. frainetto* Ten., *Q. conferta* Ait.=*Q. frainetto* Ten., *Q. esculiformis* Schwarz=*Q. frainetto* Ten., *Q. dalechampii* Ten., *Q. congesta* Presl., *Q. apennina* Lam.=*Q. pubescens* Willd., *Q. lanuginosa* Thuill.=*Q. pubescens* Willd., *Q. pedunculiflora* K.Koch=*Q. robur* L., *Q. pedunculata* Ehrh.= *Q. robur* L., *Q. sessiliflora* Sal.=*Q. petraea, Q. robur* L., *Q. brachiphylla*=*Q. pubescens* Willd., *Q. crispata* Steven=*Q. pubescens* Willd., *Q. virgiliana* Ten.=*Q. pubescens* Willd.

Throughout the 1900s other authors were influenced by the vision of Camus and Schwarz and some interesting new proposals were carried out especially in the last two decades of the nineties. Menitsky (1984) proposed the classification of the genus into three subgenera, *Heterobalanus*, *Cerris*, and *Quercus* (Table 2.1). He classified the *ilex* oaks into the subgenus *Heterobalanus* (except *Q. suber*), the white oaks into the subgenus *Quercus*, and the rest of the species into the subgenus *Cerris*.

Nixon's classification (1993) is based on an in-depth revision of the Camus work. He merged some sections, simplifying the structure of the classification, *Cerris* was joined to *Euquercus*, in the section *Quercus*, creating a group with *Cerris* and *Ilex* and white oaks. According to the Nixon system, the genus *Quercus* was formed by two subgenera, the cycle-cup oaks (*Cyclobalanopsis*) and all remaining oaks (*Quercus*).

Govaerts and Frodin (1998) published an updated review of the classification proposed by Nixon (1993). The subgenus *Quercus* included the red oaks (sect. *Lobatae*), the intermediate oaks (sect. *Protobalanus*), and the white, *cerris*, and *ilex* oaks (sect. *Quercus*).

At the end of the twentieth century, the taxonomic studies progressively shifted from the classical morphological approach exclusively based on the biometry of morphological characters to the new molecular approach.

The first attempt to use a molecular approach to carry out a classification scheme came from Manos et al. (2001) with the publication *Systematics of Fagaceae.* They proposed a classification mainly based on the reproductive traits (morphological cladistic analysis) and the phylogenetic relationship deriving from nuclear ribosomal DNA sequences. The result was the classification of *Fagaceae* into two subfamilies: *Fagoideae* (*Trigonobalanus sensu lato*) and *Castaneoideae* (*Castanea*, *Castanopsis*, *Chrysolepis*, *Lithocarpus* and *Quercus*). In a subsequent paper (Manos et al., 2008) the authors proposed a new classification, where nuclear and chloroplast DNA was used to analyse 17 taxa representative of the phylogenetic diversity of *Fagaceae*. In this study, they proposed to divide the family into two subfamilies: *Fagoideae* and *Quercoideae*. The *Fagoideae* subfamily included only the genus *Fagus*, while all the other genera were included in subfamily *Quercoideae*, as shown in the schemes of figure 2.1.

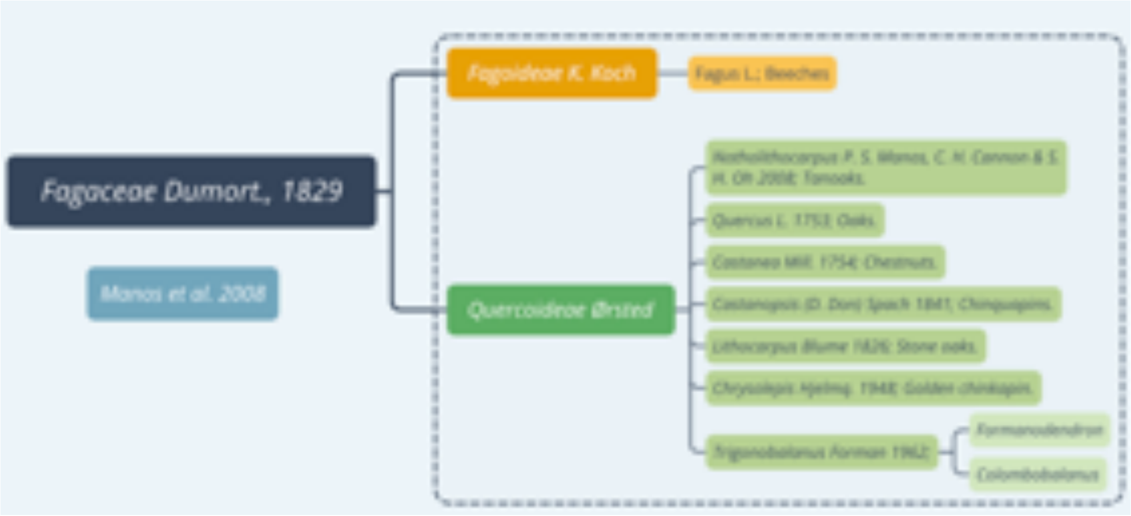

Fig. 2.1. Classification of the *Fagaceae* according to Manos et al. (2008).

From Manos et al.'s (2008) classification on, other studies have been published regarding the *Fagaceae* family and, especially, the *Quercus* group (Hubert et al., 2014; Hipp et al., 2014, 2015, 2018).

Hipp et al. (2015) proposed a new systematic tree based on a next-generation sequencing (RADseq) dataset. According to this study, the genus *Quercus* was characterised by two groups, reciprocally monophyletic. These groups were formalised into two subgenera with eight phylogenetic lineages (Hubert et al., 2014; Hipp et al., 2015). This framework broadly corresponds to the morphological groups originally hypothesised by Trelease (1924) and slightly modified by Menitsky (1984).

Denk et al. (2017) proposed a revised sectional classification of oaks, where the genus was considered as composed of two subgenera *Quercus* and *Cerris*, with five and three sections respectively (Figure 2.2)

Fig. 2.2 New Infrageneric Classification of the Oaks according to Denk et al. (2017).

In the light of this new revision, all the European oaks flow in the two subgenera *Cerris* and *Quercus*, and in the Sections *Cerris*, *Ilex*, and *Quercus* (Table 2.1). A new phylogeny investigation about global patterns of oak diversity was subsequently carried out using fossil data and restriction-site associated DNA sequencing (RAD-seq) (Hipp et al., 2019). Through this work new chronological data and information about the splitting of the lineage of oaks became available. In this work, the authors put to argue that the European clade[10] called *Roburoid* in which all the white oaks are included represents a taxa group that is difficult to diagnose due to a phenomenon of morphological and ecological convergence. Hipp et al. (2019) state that, *Roburoid* shows a *'differentiation within clades commonly reflects ecological and climatic niche evolution along with morphological adaptations (e.g. from deciduous large lobed leaves to small, brevideciduous, unlobed leaves)'*.

Very recently, Kremer et al. (2019) have proposed an interesting vision about the processes that led to the radiation and expansion of oaks around the world, based on the complementary approaches of phylogenetics, phylogeography, genomics, ecology, palaeobotany, population biology, and quantitative genetics. The authors suggest that there are four reasons for the great expansion of this group all over the world: '(1) *accumulation of large reservoirs of diversity within populations and species;* (2) *ability for rapid migration contributing to ecological priority effects on lineage diversification;* (3) *high rates of evolutionary divergence within clades combined with convergent solutions to ecological problems across clades; and* (4) *propensity for hybridisation, contributing to adaptive introgression and facilitating migration'*. According to this study, the complex taxonomic framework of oaks reveals a complex phylogenetic history.

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[10] Clade is defined as a group consisting of a common single ancestor and all descendants of that ancestor.

2.2 The taxonomy of oaks according to the Italian studies

In Italy, the first works about the genus *Quercus* were mainly focused on the regional and local scale. Bernardino da Ucria, in *Hortus Regius Panhormitanus* (1789), based on the Linnean classification system, describes some Sicilian species cultivated in the Hortus Regius Panhormitanus. He listed three evergreens oaks (*Quercus ilex* L., *Q. suber* L., *Q. coccifera* L.) and three deciduous oaks (*Q. robur* L., *Q. cerris* L., *Q. aegilops* L.) (Table 2.2).

In another regional report of the vegetation from Sicily, Joanne S. Presl and Carolo B. Presl (1826), listed four white oaks [*Q. robur* L., *Q. pedunculata* Will., *Q. pubescens* Willd., *Q. congesta* Presl. (described in Presl & Presl, 1822)] and two evergreens oaks (*Q. ilex* L. and *Q. suber* L.) (Table 2.2).

Table 2.2 Italian Taxonomic scheme of the regional White Oaks studies.

Author	**Categories**	**Genus**	**Species**
Ucria (1789)			*Q. robur* L.
Presl C. & Presl J (1822, 1826)			*Q. congesta* C.Presl., *Q. robur* L., *Q. pedunculata* Will., *Q. pubescens* Willd.
Tenore (1831)	Pubescent oaks (*sensu* Di Pietro et al. 2018)	*Quercus*	*Q. robur* Willd. (in nota: var. virgiliana, Nobilis, lanuginosa, Conglomerata), *Q. faginea* Lam. (nota= *Q. pubescens* Willd., *Q. faginea* Spr.), *Q. dalechampii, Q. apenninia Lam., Q. aesculus?, Q. pedunculata* Willd., *Q. brutia, Q. thomasii, Q. farnetto=Q. frainetto* Ten.), *Q. fastigiata* Lam., *Q. cerris* L., *Q. austriaca* Will., *Q. tournefortii* Willd.
Gussone (1844)		*Quercus*	*Q. fontanesii* Guss., *Q. austriaca* Willd., *Q. haliphleos* Lam., *Q. apennina* Lam., *Q. cupaniana* Guss., *Q. amplifolia* Guss., *Q. congesta* Presl., *Q. pubescens* Willd., *Q. leptobalana* Guss.

After these first studies about *Quercus* genus in Sicily, also Michele Tenore, one of the most important Italian botanists, made his contribution to the knowledge of the flora of southern Italy. In the first half of the nineteenth century, in the publications *Index Seminum* (1830) and *Flora Neapolitana* (1836), he described three white oaks species (*Q. frainetto*[11], *Q. virgiliana,* and *Q. dalechampii*) with '*loci classici'* located in central and southern Italy (Table 2.2). In 1844, the young collaborator of Tenore, Giovanni Gussone, described five new Sicilian's oaks in his *Florae Siculae Synopsis*. These species were: *Q. bivoniana* (evergreens), *Q. amplifolia, Q. cupaniana, Q. fontanesii, Q. leptobalana* (Table 2.2).

Four, out of all the oak taxa described as new by C. Presl, Tenore and Gussone, are currently accepted in the most recent checklist of the Italian flora (Bartolucci et al., 2018), namely *Q. congesta* C.Presl., *Quercus frainetto* Ten., *Q. dalechampii* Ten., *Q. leptobalana* Guss., whereas *Q. virgiliana* Ten. is considered a synonym of *Q. pubescens* Willd.

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[11] Michele Tenore reports two different epithets for this taxa. In the *Flora Neapolitana* (1831), he describes *Q. Farnetto* and adds a note at the end of the description: *Q. Frainetto* (*typographica menda sic evulgata*). *Ten. flor. nap. prod. suppl. 11. p. 69. Syn. edit. alt. p. 65.*The text is reported as has been written by the original author.

In 1854, Bertoloni, in his *Flora Italica* recognised 17 oak species as occurring in the Italian Flora (Table 2.3).

Table 2.3 Taxonomic scheme of the European White Oaks, referred only to the species of the white and pubescent oaks of Italy.

Author	***Tribe***	***Genus***	**Subgenus**	**Sect.**	**Sub-Sections**	**Species**
Bertoloni (1854)		*Quercus*				*Q. fontanesii* Guss. *Q. bivoniana* Guss. *Q. sessiliflora* Smith. *Q. thomasii* Ten. *Q. cerris* L. *Q. pubescens* Will. *Q. brutia* Ten. *Q. esculus* L. *Q. robur* L.
Parlatore (1867)		*Quercus*				*Q. robur* L. (include all pubescent oaks) *Q. farnetto* Ten.
Cesati, Passerini & Gibelli (1886)		*Quercus*				*Q. farnetto* Ten. *Q. robur* L.: - *var. sessiliflora* Sm:., *Q. aesculus* Italor., *Q. pubescens* Willd., *Q. virgiliana* Ten. *e Q. congesta* Presl; - *var. pedunculata: Q. racemosa* Lamk. *e Q. thomasii* Ten.,
Tornabene (1887)		*Quercus*				*Q. leptobalana* Guss. *Q. amplifolia* Guss. *Q. congesta* Presl. *Q. apennina* Lam. *Q. pubescens* Willd. *Q. austriaca* Willd. *Q. cupaniana* Guss.
Arcangeli (1894)		*Quercus*				*Q. cerris* L. *Q. farnetto* Ten. *Q. aegilops* L. *Q. robur* L.
Borzì (1885, 1911)		*Quercus*	*Robur*	*Robur*	Stirpe *Q. robur*	*Q. pedunculata* Ehrt., *Q. robur* Webb. (includes: β *Q. lanuginosa* Lam.: *Q. gussonei* Borzì, *Q. peduncularis* Borzì, *Q. amplifolia* Guss, *cuneata* Ten. = *Q. dalechampii* Ten., *brachyphylla* Kots.= *Q. cupaniana* Guss., *Q. congesta* Presl, *Q. leptobalana* Guss.), *Q. conferta* Kit. *Q. cupaniana* Guss. (includes: *Q. amplifolia* Guss., *Q. elliptica* Borzì, *Q. petiolaris* Borzì) *Q. toza* Bosc.

Table 2.3 Taxonomic scheme of the European White Oaks, referred only to the species of the white and pubescent oaks of Italy.

Author	***Tribe***	***Genus***	**Subgenus**	**Sect.**	**Sub-Sections**	**Species**
Lojacono (1907, 1913-1915)		*Quercus*		Sect. *Robur* Endl.	A *Pedunculatae*	*Q. pedunculata* Ehrh.
					AA *Sessiliflorae*	*Q. brachyphilla* Kotschy *Q. lanuginosa* Lam. *Q. congesta* Guss. *Q. dalechampii* Ten.=*Q. aesculus* Bert. *Q. virgiliana* Ten. *Q. minae* = *Q. pedemontanae*? *Q. amplifolia* Guss. *Q. sicula* Borzì *Q. farnetto* Ten. *Q. nicotrae*
Fiori (1923)		*Quercus*	*Robur* Rchb.			*Q. robur* L. (includes: subsp. *pedunculata* DC, subsp. *brutia* Ten., subsp. *thomasii Ten.,* subsp. *sessiliflora*, DC, subsp. *virgiliana* Ten., subsp. *dalechampii* Ten., subsp. *leptobalana* Guss., subsp. *Q. apennina* Lam.) *Q. farnetto* Ten.
Pignatti (1982)		*Quercus*				*Q. robur* L. *Q. frainetto* Ten. *Q. sicula* Borzì? *Q. petraea* (Matt.) Liebl. *Q. virgiliana* (Ten.) Ten. *Q. pyrenaica* Will. *Q. dalechampii* Ten. *Q. pubescens* Will. *Q. congesta* Presl.
Bernetti (1995)						*Q. robur* L. *Q. dalechampii* Ten. *Q. frainetto* Ten. *Q. petraea* (Matt.) Liebl. *Q. virgiliana* (Ten.) Ten. *Q. sicula* Borzì? *Q. pubescens* Willd. *Q. congesta* Presl, *Q. pyrenaica* Willd.?
Gellini, Grossoni (1997)		*Quercus*				*Q. congesta* Presl, *Q. petraea* (Matt.) Liebl. *Q. pyrenaica* Willd., *Q. dalechampii* Ten. *Q. pubescens* Will. *Q. robur* L. *Q. frainetto* Ten *Q. virgiliana* (Ten.) Ten.

Table 2.3 Taxonomic scheme of the European White Oaks, referred only to the species of the white and pubescent oaks of Italy.

Author	***Tribe***	***Genus***	**Subgenus**	**Sect.**	**Sub-Sections**	**Species**
Conti et al. (2005)		*Quercus*				*Q. congesta* Presl, *Q. ichnusae* Mossa, Bacch. & Brullo *Q. pyrenaica* Willd. *Q. dalechampii* Ten. *Q. petraea* (Matt.) Liebl. subsp. *petraea* *Q. robur* L. subsp. *brutia* (Ten.) O. Schwarz *Q. frainetto* Ten. *Q. pubescens* Willd. subsp. *pubescens* *Q. robur* L. subsp. *robur*
Pignatti et al. (2017)		*Quercus*				*Q. dalechampii* Ten.= *Q. tenoreana* Borzì; *Q. insularis* Borzì *Q. petraea* (Matt.) Liebl. *Q. frainetto* Ten. *Q. robur* L. *Q. congesta* C.Presl *Q. ichnusae* Mossa, Bacch. & Brullo *Q. gussonei* (Borzì) Brullo *Q. virgiliana* (Ten) Ten.= *Q. cupaniana* Guss *Q. leptobalanos* Guss. *Q. pubescens* Willd.= *Q. humilis* Mill; *Q. lanuginosa* Lam. *Q. amplifolia* Guss.
Bartolucci et al. (2018)		*Quercus*				*Q. dalechampii* Ten. *Q. petraea* (Matt.) Liebl. subsp. *austrotyrrhenica* Brullo, Guarino & Siracusa *Q. frainetto* Ten. Q. *petraea* (Matt.) Liebl. subsp. *petraea* *Q. robur* L. subsp. *robur* *Q. robur* L. subsp. *brutia* (Ten.) O. Schwarz *Q. congesta* C.Presl *Q. ichnusae* Mossa, Bacch. & Brullo *Q. leptobalana* Guss. *Q. pubescens* Willd. subsp. *pubescens*

Subsequently, Parlatore in *Flora Italiana* (1867) considered only two white oak entities at the rank of species (Table 2.3).

This idea was followed by Cesati, Passerini, and Gibelli (1886), who listed two species of white oaks, *Q. farnetto* Ten. and *Q. robur* L. the latter composed of two varieties: var. *sessiliflora* Sm. and var. *pedunculata* (Table 2.3).

Tornabene, in *Flora Sicula* (1887), listed eleven species, three already identified by Gussone for Sicily, whereas Arcangeli (1894) seven years later, identified twenty taxa (species and varieties) including both evergreens and deciduous entities (see Table 2.3 for the white oaks).

At the end of the nineteenth century, Borzì (1880, 1911) divided the European oaks of the subgenus *Quercus* (*sensu* Camus 1993) into two sections: *Robur* (*Lepidobalanus* Oerst.) and *Cerris* Oerst. Moreover, he added a new taxonomical key of identification with eight new '*stirpi*' (lineages). The deciduous oak material were classified into thirteen species, belonging to four lineages: *Q. robur* Will.; *Q. pedunculata* Ehrt.; *Q. conferta* Kit.; *Q. cerris* L. All the Italian white oaks were included in the lineage *Robur* (Table 2.3, see Viscosi, 2007).

More or less in the same period Lojacono (1907, 1913, 1915) ventured into the study of Sicilian oaks. Lojacono used especially two morphological traits commonly used in the diagnostic keys of *Quercus*, the length of the petiole, and the hairiness of the leaf. Based on the length of the petiole, the Sect. *Robur* has been divided into *Pedunculatae* and *Sessiliflorae* where *Q. pedunculata* Ehrh. was assigned to the first group, while all other species was assigned to the second group.

Fiori, in *Nuova Flora Analitica d'Italia* (1923), listed the oak species in the subgenus *Lepidobalanus*, sections *Robur*, *Cerris*, and *Suber*. According to this author, all the pubescent oaks were listed as subspecies of *Q. robur* L. (Table 2.3).

Camus (1936-1938) and Schwarz (1937) published two works that strongly influenced the taxonomy of oaks in the new nineties.

Camus divides the genus *Quercus* into two subgenera, *Cyclobalanopsis* Schneid. and *Euquercus* Nickel & Camus. *Euquercus* included oaks that inhabit the Northern Hemisphere and that showed the presence of free scales on the cupules. The classification proposed by Camus is based on some morphological characters such as lobes, acorns, and cupules, and the arrangement of acorns on the branches.

Camus recognised several species described in Italy, all included in Subgen. *Euquercus* Nickel & Camus. *Q. frainetto* Ten., was classified in Sect. *Mesobalanus* and Subsect. *Macrantherae,* while the other species were classified in the Sect. *Lepidobalanus*, Subsect. *Sessiliflorae* (*Q. lanuginosa* Lam. subsp. *eu-lanuginosa*: var. *leptobalanus* Lojac., var. *congesta* Presl., var. *gussonei* Borzì, var. *cupaniana* Guss., var. *virgiliana* Ten., and var. *amplifolia* Borzì). In the same Section (*Lepidobalanus*), Camus listed also *Q. lanuginosa* subsp. *dalechampii* Camus Lam. (see Viscosi, 2007).

Schwarz (1937) proposed a classification of oaks, based on differentiation into five subgenera: *Cyclobalanopsis*, *Erythrobalanus*, *Quercus*, *Cerris*, and *Sclerophyllodrys* (Table 2.1).

Pignatti in 1982 published *Flora d'Italia,* based precisely on the aforementioned Schwarz (1937) classification scheme and listed fifteen species of Italian oaks (Table 2.3). This check-list included as accepted Italian pubescent oaks taxa; *Q. pubescens* Willd., *Q. virgiliana* (Ten.) Ten., and *Q. congesta* C.Presl.

In 1995 Bernetti, in *Selvicoltura Speciale,* in addition to some well-known species, listed two other species: *Q. sicula* Borzì and *Q. pyrenaica* Willd. although as "doubtful" (Table 2.3).

Gellini and Grossoni, in *Botanica Forestale* (1997), based their work on the Schwarz (1937) classification system and listed three subgenera (*Sclerophyllodrys, Cerris, Quercus*) with seventeen taxa, including evergreens and deciduous (among these latter four pubescent oaks) (Table 2.3).

Mossa, Bacchetta, and Brullo (1998) described five species for Sardinia: *Q. congesta* Presl, *Q. virgiliana* (Ten.) Ten*.*, *Q. amplifolia* Guss*.*, *Q. dalechampii* Ten. and the new taxa *Quercus ichnusae*

Mossa, Bacchetta & Brullo. One year after Brullo et al. (1999), in a work on taxonomical revision about the deciduous oaks of Sicily, listed and described nine species: *Q. cerris* L.*, Q. gussonei* Borzì, *Q. fontanesii* Guss.*, Q. petraea* (Matt.) Liebl. (subsp. *austrotyrrhenica* Brullo, Guarino & Siracusa), *Q. congesta* C. Presl, *Q. virgiliana* (Ten.) Ten.*, Q. dalechampii* Ten.*, Q. amplifolia* Guss. and *Q. leptobalana* Guss.

Conti et al. (2005), in *An Annotated Checklist of the Italian Vascular Flora*, reported eighteen taxa of oaks composed of twelve deciduous and six evergreens.

In the most recent *Flora d'Italia* of Pignatti et al. (2017), the authors listed twenty species, six evergreens and fourteen deciduous (Table 2.2). These authors reported six pubescent oak taxa in their list; *Q. amplifolia, Q. congesta, Q. dalechampii, Q. ichnusae, Q. leptobalana, Q. pubescens*.

In *An Updated Checklist of the Italian Vascular Flora* (Bartolucci et al., 2018), seventeen taxa names were reported for the genus *Quercus,* namely four evergreens and thirteen deciduous (Table 2.3), among these reported five Italian pubescent oaks; *Q. congesta, Q. dalechampii, Q. ichnusae, Q. Leptobalana and Q. pubescens* subsp. *pubescens.*

In both these latter important and recent publication, Pignatti et al. (2017) and Bartolucci et al. (2018), one of the most reported species at the European level, *Q. virgiliana*, is not recognised as an accepted species in the Italian check-list.

According to the *World Checklist of Selected Plant Families* (Govaerts et al., 2019) the oak species currently accepted in the most recent Italian Flora and vascular plants Checklist, are considered as follows:

- *Quercus pubescens* subsp. *pubescens* Willd., Berlin. Baumz.: 279 (1796), nom. cons., is an accepted name;
- *Quercus congesta* C.Presl in J.S.Presl & C.B.Presl, Delic. Prag.: 32 (1822), is an accepted name;
- *Quercus dalechampii* Ten., Index Seminum (NAP, Neapolitano) 1830: 15 (1830), is an accepted name;
- *Quercus virgiliana* (Ten.) Ten., Fl. Napol. 5: 262 (1836) is a not accepted name;
- *Quercus ichnusae* Mossa, Bacch. & Brullo, Israel J. Bot. 47: 199 (1999), is an accepted name;
- *Quercus leptobalanus* Guss., Fl. Sicul. Syn. 2: 608 (1844) is considered a synonym of *Quercus congesta* C.Presl;
- *Quercus amplifolia* Guss., Fl. Sicul. Syn. 2: 607 (1844) is considered a synonym of *Q. pubescens* subsp. *pubescens* Willd.

2.4 Biosystematic researches on the European White Oaks: an overview

The occurrence of taxonomic white oak taxa apparently very similar each-other from a morphological, ecological and biogeographic point of view led many authors to investigate in more detail this group. From the end of the twentieth century on several papers based on morphometric and molecular analysis on *Quercus* material were carried out.

Dupouey and Badeau (1993), using a morphological classical approach, studied 80 populations (761 trees) in Lorraine (France). They suggested that by using morphological variables it is possible to distinguish the pure individuals belonging to *Q. robur*, *Q. petraea* and *Q. pubescens.* This approach worked with *Q. robur* and *Q. petraea* that would show observable diagnostic traits. While, stated the authors, inside *Q. pubescens* collective group a sort of morphological continuum with *Q. petraea 'was emerging without any clear character usable to taxonomically separate these two species'*.

Bussotti and Grossoni (1997) studied the variability of the micro-morphological characters of some European and Mediterranean oaks by the electron microscope (SEM). The sampling involved 28 oak species coming from five different European countries (United Kingdom, Spain, Italy, Cyprus, and France) and belonging to the three sections: *Quercus*, *Cerris*, and *Sclerophyllidrys*. They evidenced that some of the species investigated were easy to be distinguished and identified already using macro-morphological characters, whereas the complex *Q. robur*, *Q. petraea* and *Q. pubescens*, showed many individuals bearing 'intermediate' characters presumably deriving from hybridisation events which were very difficult to taxonomically identify and for which only the observation of micro-morphological features had a light diagnostic power.

Brullo et al. (1999), in the taxonomical revision of the deciduous oaks of Sicily, listed and described a set of entities related to the *Q. pubescens* group, based on the analysis of *'well-distinguished morphological, ecological and phenological features'*. The authors suggested that 9 species are to be recognised for Sicily by morphological traits, and these species were moreover described as regards their ecological, chorological, taxonomical and nomenclatural features.

All the aforementioned papers reported results basin exclusively on the analysis of morphological traits. One of the first works that tried to integrate molecular and morphological data in the study of the *Quercus* genus was published by Bacilieri et al. (1994). In this paper the authors investigated populations of *Quercus robur* and *Q. petraea* from north-west France establishing that there was a limited differentiation among these two species as regards genetic (allozymes) as well as their morphological, and ecological characteristics. In addition, they observed that there was not complete certainty about possible cases of hybridisation between these two species.

Subsequently, Bruschi et al. (2000), proposed a morphological and molecular study on *Q. petraea* and *Q. pubescens*. The samples were collected in central and north Italian Apennine (Umbria, Tuscany, and Emilia Romagna administrative regions). The authors argued that the micro-morphological characteristics represented the most reliable diagnostic characters for providing species-specific markers. The molecular results, however, demonstrated a low genetic differentiation between these two species, caused by a close affinity due to possible events of introgression and hybridisation.

Fineschi et al. (2002) published an interesting work focusing on possible migration routes moving from southern Italy to central Europe and how these events influenced the subsequent colonisation and current diversity pattern among the European white oaks. A total of 974 trees sampled from all Italian regions (including Sardinia and Sicily), were used for this work. More than 70% of the samples belonged to *Q. pubescens*, whereas the rest of the samples belonged *Q. robur*, *Q. petraea*, and *Q. frainetto*. Unexpectedly, the highest value of total genetic diversity emerged in Sardinia and central Italy and the lowest values in southern Italy and Sicily. Italian haplotypes showed more similarity with the populations located in the paleogeographic Sicilian and Balkan refugia. According to the authors, this evidence testified a great migration from the Balkans to the Italian peninsula via the Adriatic bridges occurring during the Quaternary cold periods. Whereas the Apennines have played a role in the western and eastern distribution of the haplotypes along the Italian coasts.

Borazan and Babac (2003) provided an overview of the white oaks' variability in Turkey based on their morphological traits. In this work, 291 oak samples collected at different altitudinal belts from 12 different population stands were used. The PCA showed a pattern of leaf variation as evidence of hybridisation among four critical species *Q. pubescens*, *Q. virgiliana*, *Q. petraea*, and *Q. robur*. These species showed a morphological continuum from smaller *Q. pubescens* leaves to relatively larger *Q. virgiliana*, *Q. petraea*, and *Q. robur* leaves. Also, PCA projections demonstrated that what they referred to *Q. virgiliana* was grouped in the form of a set of individuals showing intermediate morphological features between those of *Q. pubescens*, *Q. petraea*, and *Q. robur*. The final hypothesis of Borazan and Babaç (2003) is that *Q. virgiliana* had to be considered a hybrid between *Q. pubescens* and *Q. petraea*, and this was in accordance to how already reported by some other authors such as Samuel et al. (1995), Bussotti and Grossoni (1997) and Bruschi et al. (2000). These results questioned what was reported in Hedge and Yaltırık (1982) for the Turkish oaks where it was proposed that *Q. virgiliana* and *Q. pubescens* could be considered as separated species on the basis of some typical morphological traits such in particular *'petiole width, leaf largeness, size and shape of the lobes, and occurrence of a fruit short peduncle'*.

Jerše and Batic (2007), performed a morphological analysis on eight populations of *Q. pubescens,* coming from continental and sub-Mediterranean areas of Slovenia. The authors demonstrated that there were significant differences between individuals within the same populations and among the different populations. However, no one evidence of distinguishable characters for putative *Q. virgiliana* individuals were found, and this led the authors to exclude the presence of *Q. virgiliana* in Slovenia as well as, suggested in papers of the other authors for Croatia (Škvorc, 2003; Škvorc et al., 2005).

Another attempt to resolve the issue of the presence of *Q. virgiliana* in the eastern part of Europe (i.e., Romania), was proposed by Sofletea et al. (2011). This study was based on a sample size of 119 oak individuals belonging to putative *Q. pubescens* and *Q. virgiliana* populations coming from Dobrogea (southeastern Romania) and Banat (southwestern Romania). The results suggested that all the individuals sampled were to be classified as *Q. pubescens*, on the basis of sessile or nearly sessile fruits and some specific leaf descriptors. The authors argue that no typical *Q. virgiliana* specimens seem could be hypothesised as occurring in the study area.

The genetic and morphological variability of the *Q. pubescens* group in a west-central Romania forest was studied also by Curtu and Gailing (2007a). Analysing a total of 269 individuals coming from a natural community formed by *Q. robur*, *Q. petraea*, *Q. pubescens*, and *Q. frainetto*, the authors were able to assign to pure species ~94% of the sampled individuals based on the leaf pubescence and various other morphological traits considered typical of the pure species. Besides, the molecular analysis highlighted that the natural rates of hybridisation among *Q. robur*, *Q. petraea*, *Q. pubescens*, and *Q. frainetto* occurring in this Romanian location were moderately low and that it was possible that the occurrence of intermediate phenotypic features, could be due to genetically intermediate individuals more than hybridisation.

Using both the morphological traits of the leaves and the RAPD-PCR molecular technique, Franjic et al. (2006), tried to assess the taxonomic status of *Q. pubescens* in Croatia, analysing 100 individuals of 10 populations representative of the different ecological condition occurring in the Croatian territory. Results highlighted differentiation between the southern pubescent oak populations collected along the coastline (Mediterranean) and those occurring in the inner and northern (Continental) areas.

Although confirming a high morphological variability in the populations of *Q. pubescens* this morphological/molecular analysis did not support the hypothesis that *'more than one species occurring among the pubescent oak population of Croatia'*. The authors, however, hypothesised that the southern populations could be formed by pure *Q. pubescens* samples, while some northern ones the result of introgression with *Q. petraea*.

In another Croatian study, Trinajstic (2007) analysed the morphological differences between putative populations of *Q. pubescens* and *Q. virgiliana.* He concluded that these two entities are distinguishable from each other by leaves, cupules and bark traits, as well as by the arrangement of flowers and by the time of appearance of their cupule.

Fortini et al. (2009) investigated the micro-morphological and molecular features of five related species - *Q. robur*, *Q. petraea*, *Q. frainetto*, *Q. pubescens*, and *Q. virgiliana* - from southern and central Italy. A total of 33 specimens for micro-morphological analysis and 42 for molecular analysis were used. Results showed that the five groups identified based on morphological markers corresponded with the groups obtained by molecular analysis. However, the specimens classified prior as *Q. virgiliana*, at the end of the analysis was found to be very closely related to *Q. pubescens*.

In the same period Viscosi et al. (2009), suggested that using the genetic assignment of species, and subsequently using leaf morphological data, it was possible to discriminate at least among the following entities: *Q. robur*, *Q. pubescens*, *Q. petraea*, and *Q. pyrenaica*. The authors sampled 817 oak individuals to measure 19 leaf variables, while 10 microsatellites loci were used to genotype the individuals. In addition to allowing correct assignment of samples to putative species, this approach permitted the identification of some hybrid forms.

Especially in the last ten years, unlike what one might think, the *Q. pubescens* group has been at the centre of interdisciplinary studies focused on not only the molecular analysis.

The research of Ballian et al. (2010) was focused on the genetic variability (chloroplast DNA) of some Balkan populations coming from Bosnia-Herzegovina, Montenegro, Serbia-Kosovo, Croatia, Macedonia, and Albania. This study, using 117 individuals from 36 different populations, highlighted a great variability from pubescent oaks along the Balkan area. The authors suggested that existing differences between populations were to be related to postglacial migration events. Only one haplotype, out of the four originating from the Balkan refugia, was found to occur throughout the entire research area. Two haplotypes exhibited an amphi-adriatic distribution and the other two were found to be more geographically restricted.

According to Coldea et al. (2010), good discrimination of *Q. pubescens* and *Q. virgiliana* would be possible observing the fruit morphology, the scales at the base of the cupule and the leaf morphology, together with information from the ecological features, and distribution area. Accordingly, these authors considered *Q. virgiliana* (Ten.) Ten. and *Q. pubescens* Willd. as valid names for two different taxonomical entities.

Viscosi et al. (2011), based on 309 samples by 37 populations of European white oaks coming from southern and central Italy and Austria arranged a morphological statistical analysis using morphological traits of the young twigs, buds, leaves and fruits. Results showed the presence of five morphological clusters that corresponded to five species, three of them, *Q. frainetto*, *Q. petraea* and *Q. robur,* were found to be clearly discriminated, while *Q. virgiliana* and *Q. pubescens* were found to be strictly related, but at least still partially discernible. On the contrary the identification of putative *Q. dalechampii* was unclear.

One year after Viscosi et al. (2012) combined a genetic and morphometric approach to study leaf shape and size variability among three sympatric and inter-fertile white oak species (*Q. frainetto*, *Q. petraea*, and *Q. pubescens*) sampled in a mixed forest in central Italy. The analyses performed emphasised the role of the multivariate ordination of shape variables especially in highlighting differences between *Q. frainetto* and the other two species, whereas the canonical variate analysis allowed to identify a significant differentiation among all the species investigated.

The taxonomic status and genetic diversity of large populations of pubescent oaks in Germany and Poland have been assessed using DNA markers. Over 14,500 individuals of all ages were identified by Kätzel et al. (2014). The molecular analysis has pointed out that: *'DNA markers were applied in most of the populations in order to assess their taxonomic status and genetic diversity. Diverging values of genetic diversity and high genetic differentiation compared to populations of Q. petraea and Q. robur indicate that genetic drift has strongly affected genetic structures due to reproductive isolation and small effective population sizes'*. Through genotype-based clustering methods, the authors have been able to demonstrate that only three German and one Polish population were formed by 'pure' individuals belonging to *Q. pubescens* whereas two further stands were classified as 'almost pure' *Q. pubescens*. Most of the other analysed populations were composed by a large number of putative hybrids or sessile oak individuals.

Fortini et al. (2015a) proposed an approach based on a micro-morphological study of the trichomes and stomatal traits about for sympatric oak species belonging respectively to *Q. frainetto*, *Q. petraea*, and *Q. pubescens*. The sampling performed in the Molise administrative region included a total of 268 oak trees. In this work, the authors demonstrated that the pattern and quantitative values of micro-morphological trait examined had an important role in identifying hybrids and pure species.

Fortini et al. (2015b) made another attempt to study this critical group by applying a multidisciplinary method based on macro-morphological leaf traits and genetic assignments. This study is based on measurement of leaves randomly collected in nine stands of the natural mixed forest of Monte Vairano (Molise region), formed by *Q. frainetto*, *Q. petraea*, and *Q. pubescens*. A total of 265 trees were investigated. The authors argued that in this way it was possible identify pure individuals belonging to *Q. frainetto*, *Q. petraea*, and *Q. pubescens*. The variables used with success in this study were basal leaf shape, petiole ratio, petiole length, a number of intercalary veins, pubescence of the petiole, leaf area, number of lobes, lamina length, and percentage of venation. Among these three species studied, *Q. pubescens* exhibited the main degree of leaf trait variability respecting *Q. petraea* and *Q. frainetto*.

Based on the hypothesis that incomplete reproductive barriers in sympatric conditions can increase the likelihood of hybridisation and genetic diversity within species, Antonecchia et al. (2015), using 11 microsatellite markers, the Expressed Sequence Tag (EST-SSRs), tried to assess genetic diversity in a population of closely related oaks (*Q. frainetto*, *Q. petraea*, and *Q. pubescens*) in central Italy (Molise administrative region). The results showed that three of the most important white oaks in central-southern Italy (*Q. frainetto*, *Q. petraea*, and *Q. pubescens*) exhibited significant genetic differences and a higher level of gene flow. Several woods-patches of the study area were made up of hybrids, mainly between *Q. petraea* and *Q. pubescens*, whereas they were rare the hybrid individuals between *Q. petraea* and *Q. frainetto*. According to this work, the *Q. frainetto* populations from central Italy exhibited a low degree of diversity when compared with those exhibited by the other two oak species.

Crãciunesc et al. (2016) published a work in which a set of non-genic SSRs (simple sequence repeats) and EST-SSRs were used to evaluate genetic diversity among *Q. robur* and *Q. petraea*. A total of 192 individuals were sampled in four different stands of central and northern Romania. The results showed that *Q. robur* and *Q. petraea* exhibited similar values of genetic diversity, that only two, out of the 10 loci used, showed high F_{st} values and that genetic distances were more significant between species than that occurring between populations within the same species (Crãciunesc et al., 2016).

Rellstab et al. (2016), reaffirmed that the complex taxonomic condition of the European white oaks was resolvable by combining two methods - morphological and genetic. These authors sampled 71 oak populations from and found that the individuals belonging to *Q. robur* was easily distinguishable from those of *Q. petraea* and *Q. pubescens.* However, whereas these latter two species were found to be abundantly overlapping as regarded morphological and genetic features. Furthermore, the authors claimed that molecular analysis could give clearer results than morphological ones.

Di Pietro et al. (2016), proposed a morphological study about a large population of oaks in Apulia (Italy), using 367 trees, 4,254 leaves, and 1,120 fruits, collected in 24 stands. The authors demonstrated that among 25 morphological characteristics observed, only leaf and fruit 'size' and fruit petiole length emerged as slightly discriminating characteristics. The work aimed to find out whether in that oak population in Apulia there were one or more different entities related to the *Q. pubescens* s.l (e.g. *Q. pubescens, Q. virgiliana* and *Q. dalechampii* as from the taxonomic and phytosociological literature). The results suggested that the presence of more than one species belonging to the *Q. pubescens* complex in Apulia was improbable. The authors have also tested their hypothesis by comparing a genetically pure *Q. pubescens* population coming from the Molise administrative region and they found that the individuals of this population mixed with those of all the Apulian populations.

Musarella et al. (2018), performed a similarity analysis of a group of pubescent, deciduous and evergreens oak species, based on fractal dimension. Their study analysed samples coming from *Q. robur* subsp. *brutia*, *Q. cerris*, *Q. congesta*, *Q. crenata*, *Q. ilex* subsp. *ilex*, *Q. suber* and *Q. virgiliana* collected in the Calabria region. The values of means and medians of fractal dimension (FD) well described the distance between the deciduous and evergreens populations whereas distance values resulted very low when observed within the deciduous group only.

2.5 Final Considerations

The long taxonomic history of oaks and the whole *Fagaceae* family has found a landing point with the advent of molecular biology. With these techniques, several issues, linked to the subfamilies and infra-generic relationship, have been resolved. Nowadays, there is a quite solid classification in which it is possible to identify the well-established species and reconstruct not only the kinship relationships but also the periods of the splitting of different infra-generic groups.

The same cannot be said of European oaks, especially for many taxa listed in section *Quercus*. In particular, the situation of the Italian pubescent oaks, which, despite their long history (beginning in the nineteenth century), has not found its unambiguous taxonomic classification yet.

For many species of the Italian pubescent oaks, it is still not possible to highlight accepted diagnostic features that would legitimise the status of the species.

However, the biosystematic analysis that I have carried out in this PhD work offers some aspects that allow us to contribute to solving some intricate taxonomic question as regards the southern Italian white oaks populations.

At present *Quercus pubescens* Willd is the only pubescent-oak taxon universally accepted in all the Floras and checklists of Europe. This species which is known of being well adapted to drought, is widespread throughout the Mediterranean basin, from Sicily to the North Atlantic coast of France, to the coasts of the Black Sea, Crimea, and the Caucasus (Pasta et al., 2016). This huge area of distribution is the result of a good adaptive capacity, which is also reflected in the high phenotypic variability. According to some authors, this is the reason why, in different regions of Europe, but especially in Italy, several phenotypic forms of pubescent oaks have been described (Trehane, 2007).

I have observed that two main approaches have been used in the last 25 years to study the systematic and taxonomy of white and pubescent oaks - the morphological method, based on a morphometric model of classical traits, and the molecular analysis based on allozymes, EST-SSR, RAPD-PCR, chloroplast DNA.

Two of the most studied species of pubescent oaks in the biosystematics works are *Q. pubescens* s.l. and *Q. virgiliana* (Ten.) Ten., although the degree of consideration of these two species is depending on the author of the work. *Q. virgiliana* is not considered an accepted species by Govaerts et al. (2019). Nonetheless, some previous works gave credit to the existence of diagnostic traits usable to separate *Q. pubescens* from *Q. virgiliana* (Hedge & Yaltrik, 1982; Brullo et al., 1999; Trinajstic et al., 2007; Fortini et al., 2009; Coldea et al., 2010; Viscosi et al., 2011). In other cases, the authors suggested that the putative individuals of these two species exhibited a continuum of morphological traits between the two related species (Bussoni & Grossoni, 1997; Borazan & Babac, 2003; Sofletea, 2011; Musarella et al., 2018).

Many works suggest that the difficulty of determining some taxa is due to the occurrence of hybrid forms among white and pubescent oaks, such as *Q. robur*, *Q. petraea* and *Q. pubescens* (Dupouey & Badeau, 1993; Bussotti & Grossoni, 1997; Fortini et al., 2009, 2013; Viscosi et al., 2009, 2011, 2012; Antonecchia et al., 2015; Di Pietro et al. 2016; Rellstab et al., 2016; Musarella et al., 2018).

The existence of unclear diagnostic traits is well documented, especially inside the *Q. pubescens* collective groups and for this reason, it was hypothesised that the morphological and molecular intermediate features of many pubescent oaks could be related to events of introgression and hybridisation (Dupouey & Badeau, 1993; Bussotti & Grossoni, 1997; Bruschi et al., 2000; Borazan & Babaç, 2003; Franjic et al., 2006; Curtu et al., 2007a; Fortini et al., 2013, 2015a,b; Antonecchia et al., 2015; Rellstab et al., 2016).

Nevertheless, there are species of white oaks - *Q. robur*, *Q. petraea*, and *Q. frainetto* - for which correct determination can be made using the classical morphological characteristics and molecular approaches, despite the existence of borderline cases due to gene flow phenomena, especially in the presence of individuals of *Q. pubescens* (Fineschi et al., 2002; Curtu et al., 2007a; Fortini et al., 2009; Viscosi et al., 2011).

Regarding some oak species considered typical of the flora of southern Italy, such as *Q. congesta*, *Q. leptobalana*, *Q. dalechampii*, *Q. amplifolia* and *Q. ichnusae*, there is no general agreement about their consistency. This is particularly true as regards species such as *Q. leptobalana* and *Q. amplifolia*.

The reason for this complex taxonomic situation is probably linked to the lack of reproductive barriers among white and pubescent oaks. This could determine a high degree of hybridisation and introgression in sympatric populations.

The lack of genetic isolation, between white and pubescent oaks, could be linked at the long and complex history of this group, which has gone through a large number of environmental upheavals. These ecological changes could have favourited a constant gene flow (and low reproductive isolation), especially during the Quaternary Ice Age (see Petit et al., 2003).

The habitat fragmentation, the destruction of forests and the artificial planting, determined by the human activities in the last 11,000 Bp, combined to some other ecological causes, could have stimulated the re-emergence, or a new evolution of some morphological traits. These responses could be the sign of springing of new micro-evolution episodes (Briggs, 2009).

Often some authors have highlighted that some diagnostic characteristics vary continuously between populations of white and pubescent oaks, especially among *Q. petraea*, *Q. pubescens* s.l. and all other Italian pubescent oaks.

This lack of clear diagnostic traits makes it impossible to highlight discontinuity in the intervals between morphological variables justifying the passage from one species to another (Borazan & Babaç, 2003; Franjic et al., 2006; Curtu et al., 2007a; Fortini et al., 2013, 2015 a, b; Kätzel et al., 2014; Antonecchia et al. 2015; Wellstein & Spada, 2015; Crãciunesc et al., 2016; Rellstab et al., 2016).

Introduction to the Morphological variability

The morphological approach is the most used and accepted method for describing, naming and classifying life on this planet. The description of organisms based on their phenotypical traits has always been considered a secure method to identify and name them. Nevertheless, this classical method seems not to work completely with some particular groups of organisms, especially in the plant world. Taxonomists call groups that do not permit a clear taxonomic ranking '*critical*'.

The classification of such critical groups appears to be a complex issue. A different and more modern approach to a morphological study is the morphometry. Morphometry is based on the quantification of the morphological features and the elaboration of data through a statistical approach. It is possible to use numerical, categorical (qualitative) and derived variables to evaluate the presence of a discontinuity in the data-set that can lead to emerging of good diagnostic traits (Elewa & Elewa, 2010).

The long taxonomical history of white oaks is revealed by the many attempts to describe a species using a set of clear diagnostic characters. However, there is still considerable disagreement concerning the taxonomic frameworks of this genus (Camus, 1936-54; Schwarz, 1937, 1964; Nixon, 1993; Govaerts & Frodin, 1998). According to some authors, the phenotypic variability of this group is due to the low efficiency of the reproduction barriers that generate a high level of interaction between different genetic pools. This latter phenomenon seems to be very frequent in the natural populations of the genus *Quercus* (Burger, 1975; Spellenberg, 1995; Gonzàles-Rodrìguez et al., 2004; Kremer & Hipp, 2019). The high level of gene flow among the different populations of oaks results in a wide spectrum of intermediate morphological characters (Gellini & Grossoni, 1997; Curtu et al., 2007a, 2013; Gailing et al., 2014; Sullivan et al., 2016; Castillo-Mendoza et al., 2019; Jurkšienė et al., 2019). This condition is persistent mainly in the sympatric species of pubescent oaks of southern Italy. Here, the overlapping distribution of several entities, e.g. *Q. pubescens, Q. petraea, Q. robur* and maybe *Q. frainetto* too during the cold periods of the Quaternary led to a high level of hybridisation which is probably the cause of the high degree of morphological variability (and consequence taxonomical splitting) that we observe at present (Petit et al., 2003).

Morphological and molecular analyses performed on some species of white oaks appear to work well, discriminating, for example, between *Q. robur/Q. farnetto*, or *Q. petraea /Q. pubescens* (Fineschi et al., 2002; Curtu et al., 2007a; Fortini et al., 2009; Viscosi et al., 2011). However, when the same procedure, are used for the group of pubescent oaks, it becomes impossible to select morphological traits or molecular divergences able to characterize the different putative taxa included in the *Q. pubescens* collective group. For this reason, the number of Italian pubescent oaks, classified at the rank of species, is variable, depending on which flora or checklist we are considering (Di Pietro et al., in press). According to Pignatti et al. (2017) there are six pubescent oak taxa (*Q. pubescens, Q. dalechampii, Q. congesta, Q. leptobalana, Q. amplifolia, Q. ichnusae*) whereas Bartolucci et al. (2018) consider only four of them since *Q. virgliana, Q. leptobalana, Q. amplifolia* are considered synonyms.

What is certain is that the debate around these issues is still open.

Chapter 3. A multivariate morphometric analysis of phytognostic traits in southern Italy and Sicily pubescent-oaks[12]

Romeo Di Pietro[1], Antonio Luca Conte[2], Piera Di Marzio[2], Lorenzo Gianguzzi[3], Giovanni Spampinato[4], Orazio Caldarella[3], Paola Fortini[2]

[1]Department PDTA, University of Rome Sapienza, 00196 Rome, Italy.
[2]Department of Bioscience and Territory, University of Molise, 86090 Pesche (IS) Italy.
[3]Department of Scienze Agrarie e Forestali, University of Palermo, Italy.
[4]Department of AGRARIA, Mediterranean University of Reggio Calabria, Italy.

✉ Corresponding author, Romeo Di Pietro: romeo.dipietro@uniroma1.it

Abstract

Species identification within the collective group of *Q. pubescens* represents a well-known taxonomic node among European botanists. Some of the specific pubescent-oak binomials currently accepted in various European floras and checklists have been described in Sicily and southern Calabria. Accordingly, several species belonging to the pubescent-oaks group (*Q. pubescens, Q. virgiliana, Q. dalechampii, Q. congesta, Q. amplifolia and Q. leptobalana*) are reported in both taxonomical and phytosociological literature. In order to verify whether a diverse set of morphological characters was possible to be associated with each these different taxa, thirteen natural populations of pubescent-oak from Sicily and southern Calabria were sampled. A total of 391 trees, 3887 leaves and 1047 fruits were collected. Overall, 28 morphological characters of oak leaves and fruits were statistically analysed using univariate and multivariate analysis procedures. The results showed that neither the groups of morphological diversity identified by cluster analysis nor those obtained by our expert identification, through the use of analytical keys matched with the current taxonomical frameworks as proposed by the most recent floras and checklists. Nearly all of the morphological characters considered showed a more or less continuous trend of variation both within and among populations. Only the sizes of leaves and fruits and the lengths of the fruit petioles showed a minimal discriminating power as far as only those groups that emerged from the cluster analysis. In the light of these facts, it seems unlikely that more than one species of pubescent-oak occurs in Sicily and southern Calabria.

Keywords: Diagnostic morphological characters, Nomenclature, *Quercus*, Taxonomy, southern Europe.

Running title: Phytognostic traits in southern Italy and Sicily pubescent-oaks.

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[12] This chapter has been submitted as an article on Folia Geobotanica (Springer). And in this thesis has reported in the same model submitted at the journal. The tables and figures are related to this part of the text and not to the general numbering followed for the other chapters of the thesis.

Introduction

The majority of the southern Europe forest communities are characterised by the dominance of species belonging to the genus *Quercus* L. The genus counts about 435 species in the world (Denk et al., 2017) of which a little more than one hundred occur only in Europe. Classifications based on molecular studies (Manos et al., 2008; Denk, 2010; Hubert, 2014; Hipp et al. 2015, 2019) have supported the hypothesis that the *Quercus* genus is divided into two monophyletic groups, which correspond to two different subgenera, Subgenera *Quercus* (composed of five Sections) and Subgenera *Cerris* (composed of three Sections) (Denk et al., 2017). Both of these subgenera are widespread in Italy, where they include both evergreens and deciduous species. The total number of oak species occurring in Italy is not known exactly, because this number has been subject to continuous changes over time. This number ranges between 9 and 15, depending on which flora or checklist we are considering. This wide discrepancy about the number of accepted oak species names is due, almost exclusively, to the descriptive and interpretative nebulosity that still characterises the collective group of *Q. pubescens* (Table 1).

Table 1 List of the oak taxa belonging to the collective group of *Q. pubescens* described for Italy according to some of the major floras and checklists.

	Q. amplifolia **Guss. 1844**	***Q. congesta*** **C.Presl, 1822**	***Q. dalechampii*** **Ten. 1830**	***Q. ichnusae*** **Mossa, Bacch. and Brullo 1999**	***Q. leptobalana*** **Guss. 1840**	***Q. pubescens*** **Willd. 1796**	***Q. virgiliana*** **(Ten.) Ten. 1836**
Flora d'Italia (Pignatti 1982)	synonym of *Q. virgiliana*	accepted	accepted		synonym of *Q. congesta*	accepted	accepted
Flora Europaea (Schwarz 1993)	-	accepted	accepted		-	accepted	-
International Oak society (Trehane 2007)	-	accepted	accepted		-	accepted	-
Atlas of European tree (De Rigo et al. 2016)	-	-	-		-	accepted	-
Flora d'Italia (Pignatti 2017)	accepted	accepted	accepted	accepted	accepted	accepted	accepted
Checklist of the Italian Vascular Flora (Bartolucci et al. 2018)	synonym of *Q. pubescens*	accepted	accepted	accepted	accepted	accepted	synonym of *Q. pubescens*
Global Tree Search (GlobalTreeSearh 2019)	-	accepted	accepted		-	accepted	-
Euro+Med Plantbase (http://ww2.bgbm.org/ EuroPlusMed/)	synonym of *Q. pubescens*	accepted	accepted		synonym of Q. congesta	accepted	synonym of *Q. pubescens*
World Checklist of Selected Plant Families (WCSP 2019)	synonym of *Q. pubescens*	accepted	accepted		synonym of *Q. congesta*	accepted	synonym of *Q. pubescens*

The classification systems of oaks are normally based on the observation and measurement of a variable number of morphological traits of leaves and acorns, such as the degree of pubescence, its persistence on twigs and leaves, the dimensional traits of the leaf, the numbers, shapes and depths of the leaf lobes, length of petiole, type of the margin and the surface of the cupule, among others. This classification system, if efficient to provide a first rather clear separation among well-known white oak species and long established names (e.g. *Q. robur* L., *Q. frainetto* Ten., *Q. petraea* (Matt.) Liebl., *Q. pubescens* Willd.), goes into crisis when trying to discriminate inside some particularly critical groups (e.g. the collective groups of *Q. pubescens* or *Q. petraea*) where, especially in the first group, a more or less continuous pattern of morphological variation is observed in about all the diagnostic characters. This phenomenon, more common in the oaks than in the other genera of European trees, is generally ascribed to a complex pattern of introgression and hybridisation and to the convergent morphological evolution processes that have characterised oaks (in particular the whole group of white oaks) over time (Oh and Manos, 2008; Kremer et al., 2012; Denk et al., 2017). These hybridisation events among white oaks especially occurred in those areas, such as southern Italy, where the thermophilous woody flora took refuge during the cold periods of the Quaternary (Follieri et al., 1989; Petit et al., 2006; Magri et al., 2007; Leroy et al., 2019).

Moving on to the nomenclatural issues, even excluding all those binomials described in the past centuries for southern Italy and which are now reported almost everywhere as synonyms of other oak names, such as *Q. apennina* Lam., *Q. cupaniana* Guss. and *Q. tenoreana* Borzì, among others (cf. Borzì 1905, 1911; Lojacono-Poiero 1907, 1913-15), a high number of pubescent-oak species is still reported for Italy in the most recent floras and checklists. According to Brullo et al. (1999) and Pignatti et al. (2017-2019) the occurrence of *Q. pubescens* Willd. should be excluded from southern Italy where it would be replaced by other deciduous oak thermophilous species having a strictly steno-Mediterranean distribution [e.g. *Q. virgiliana* (Ten.) Ten., *Q. congesta* C. Presl., *Q. dalechampii* Ten., *Q. leptobalana* Guss. and *Q. amplifolia* Guss]. However, not all the above-mentioned taxa are taken in the same consideration by oak taxonomists. *Q. pubescens* is universally considered as a binomial valid throughout Europe, except for Spain where it is substituted by the name *Q. humilis* Lam. (Amaral Franco, 1990). As regards the other taxa, some of them are accepted species in the majority of European floras and checklists (e.g. *Q. congesta* and *Q. dalechampii*); others (e.g. *Q. virgiliana*) are reported for many southeastern-European countries, but in many cases, they are considered synonyms as well as *Q. leptobalana* and *Q. amplifolia*, which are endemic to southern Italy. In addition to the lack of agreement on the number of white oak species names to be considered as accepted, there are delays in the updating of the taxonomical frameworks and persistent cases of "misinterpretation" that destabilise the Italian and European deciduous oak classification system. A typical example is that of *Q. dalechampii*, which although univocally typified and definitely assigned to the pubescent-oaks group (Di Pietro et al., 2012), is in some cases classified as still belonging to the *Q. petraea* collective group shown on distribution maps that mix records of pubescent and glabrous specimens (see Euro+Med 2006-2014).

Focusing on the group of pubescent oaks, several papers aiming to establish the possible occurrence of other pubescent-oak species in addition to *Q. pubescens* Willd. have been published in the last decade for southern Europe. Some of these studies were mainly based on the analysis of morphological characters but involved, in turn, geometric morphometric analyses (Viscosi and Fortini, 2011), micro-morphological leaf traits (Scareli-Santos et al., 2007; Fortini et al., 2009; Panahi et al., 2012; Scareli-Santos et al., 2013), fractal analysis of leaf morphology (Musarella et al., 2018) and molecular data (Curtu et al., 2007a,b, 2015). As far as southern Italy is concerned, two recent papers (Di Pietro et al., 2016; Di Pietro et al., 2020) analysed 24 southeastern Italian populations of pubescent oaks from a macro-morphological and molecular standpoint. Both of these studies established that in this area there was neither morphological nor molecular evidence that could confirm the occurrence of more than one pubescent-oak species, despite the floristic and phytosociological literature reporting four. In this study, we analysed the pubescent-oak populations of Sicily Island and southern Calabria, this area being crucial for the taxonomical and nomenclatural diversity of deciduous Mediterranean oaks. In fact, Sicily and southern Calabria hosts the highest number of pubescent-oak species currently reported for the Italian Peninsula (Pignatti et al.,2017-2019; Bartolucci et al., 2018), together with the majority of their '*loci classici'* and the highest number of pubescent-oak forest phytosociological associations (Blasi et al., 2004). In addition, these territories were refuge areas, both primary and secondary, during glacial periods and constitute an important reservoir of genetic diversity that has been preserved over time despite the strong anthropic pressure exerted by the civilisations that have taken place over the last three millennia (Médail and Diadema, 2009). This paper aimed to verify whether this high nomenclatural and coenological diversity, which involves several pubescent-oak taxa, was supported by sufficient taxonomic discrimination of those taxa based on i) the result of objective statistical analyses; ii) sets of unambiguous and identifiable diagnostic morphometric characters.

Materials and Methods

Sampling

The research was carried out through a wide sampling of 13 pubescent-oak populations throughout Sicily and southern Calabria. Based on the floristic and phytosociological literature, these populations included the following oak taxa: *Q. congesta*, *Q. dalechampii*, *Q. leptobalana*, *Q. virgiliana* and *Q. amplifolia*. The sites of the collection were selected from the entire deciduous oak woodlands bioclimatic belt, from the sea level to the lower montane belt (Gianguzzi et al., 2016) and different types of lithological substrates (Fig. 1 and Table 2).

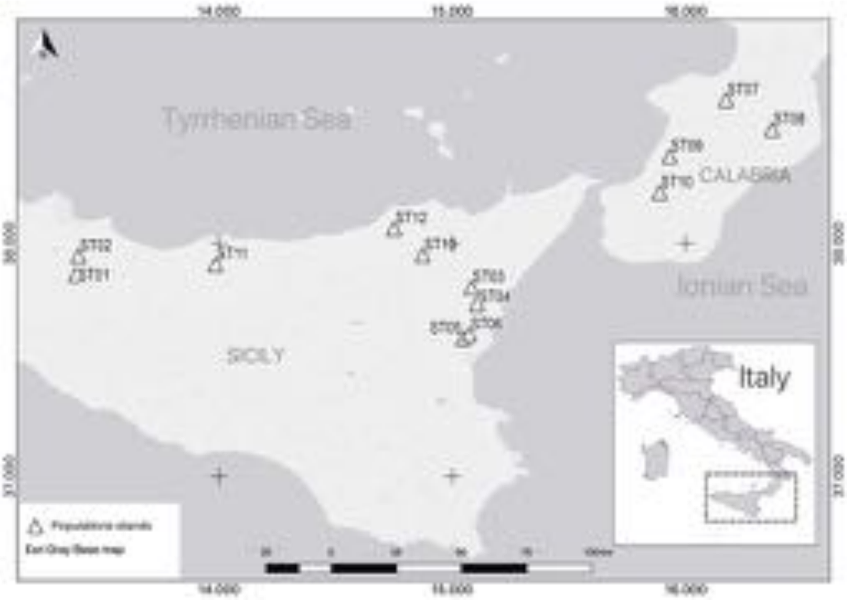


Fig. 1 Spatial distribution of the 13 pubescent-oak populations sampled in the study area

Where possible, priority for the collection was given to those pubescent-oak populations located in the proximity of the '*loci classici*' of the species in question and to those already described from a syntaxonomic point of view through already published phytosociological tables (Table 3). The collection of leaves and fruits were taken from 27 to 33 adult trees in autumn 2017 and 2018. Up to three branches were selected randomly in the middle-upper part of the crown. Voucher specimens are stored in the Herbarium of the University of Molise (IS).

Ten leaves per tree specimen were pressed, dried and scanned using an Epson GT-15000 scanner with a resolution of 600 dpi, with the abaxial surface facing upwards, and measured with ImageJ (Rasband, 1997-2007).

In total, 17 morphological characters were assessed (Table 4): Seven-dimensional variables (leaf area, leaf perimeter, leaf lamina length, petiole length, the width of the major lobe, sinus width, widest point) are direct and were measured as already described in Fortini et al. (2015). Five were derived variables (leaf compactness, obversity, lamina/petiole ratio, lobe depth ratio, lobe width ratio) and were obtained based on the measures of the direct dimensional variables. One variable (number of lobes on the left side) is numerical (counted), and one other (scoring of the basal shape of the lamina) was evaluated following the Kremer scale (2002). Finally, three variables (abaxial and adaxial laminar pubescence as well as twig pubescence) were observed on voucher specimens using Kissling's scale (Kissling, 1980).

Table 2 Geographical coordinates and descriptions of the sites of collections (ST) according to Brullo et al. (2009) and Gianguzzi et al. (2016).

Station ID	Sample size	Site of collect. (Administrative province)	Coordinates D° M' S"	Italian Region	Dominant taxon	Altitude m a.s.l.	Substrate
ST01	29	Bosco Ficuzza (PA)	37°51'55.98" N 13°23'13.68" E	**Sicily**	*Quercus leptobalana*	**919**	Flysch
ST02	31	Santuario Marineo (PA)	37°56'50.59" N 13°23'43.71" E	**Sicily**	*Quercus virgiliana*	**459**	Clays on carbonate matrix
ST03	30	Chalet delle Ginestre (CT)	37°48'40.30" N 15° 4'56.26" E	**Sicily**	*Quercus congesta*	**1298**	Ancient Etna lava flows
ST04	30	Mare-Neve over Fornazzo (CT)	37°44'19.60" N 15° 6'18.64" E	**Sicily**	*Quercus dalechampii*	**900**	Ancient Etna lava flows
ST05	29	Monte Ceraulo (CT)	37°35'32.67" N 15° 2'42.61" E	**Sicily**	*Quercus virgiliana*	**538**	Ancient Etna lava flows
ST06	30	Trecastagni (CT)	37°36'29.87" N 15° 4'17.95" E	**Sicily**	*Quercus virgiliana*	**544**	Ancient Etna lava flows
ST07	27	Serre (VV)	38°36'57.93" N 16°10'17.16" E	**Calabria**	*Quercus dalechampii*	**260**	Clays
ST08	32	Serre (VV)	38°29'16.81" N 16°22'11.31" E	**Calabria**	*Quercus congesta*	**1190**	Granites

Table 2 Geographical coordinates and descriptions of the sites of collections (ST) according to Brullo et al. (2009) and Gianguzzi et al. (2016).

Station ID	Sample size	Site of collect. (Administrative province)	Coordinates D° M’ S”	Italian Region	Dominant taxon	Altitude m a.s.l.	Substrate
ST09	28	Croce Mammone (RC)	38°22’24.18" N 15°55’49.69" E	**Calabria**	*Quercus dalechampii*	**70**	Alluvial deposits
ST10	30	Piani di Carmelia (RC)	38°13’9.00" N 15°53’16.61" E	**Calabria**	*Quercus congesta*	**980**	Metamorphites
ST11	33	Piano Torre e Piano Zucchi (PA)	37°54’36.56” N 13°59’06.12" E	**Sicily**	*Quercus leptobalana*	**864**	Flysch
ST12	30	Valle del Fiume Fitalia (ME)	38°03’51.68” N 14°45’01.68" E	**Sicily**	*Quercus virgiliana*	**246**	Flysch
ST13	32	Bosco del Flascio (ME)	37°56’29.75” N / 14°52’34.38” E 37°57'07 84” N / 14°52’15.22” E	**Sicily**	*Quercus congesta*	**1170**	Flysch

Fruit measurements were made using an electronic digital calliper MAURER 93110. In total, 11 fruit variables were assessed on voucher specimens (Table 5) including four-dimensional variables (cupula length, cupula width, acorn length, acorn width), two derived variables (cupula width/ cupula length and cupula length/ acorn length) and five categorical (dimensionless) variables (regularity of cupula edge, scale shape, gibbosity, cupule internal hairiness and type of cupula edge).

Table 3 Syntaxonomic references of the oak communities occurring in the sampled areas.

Station ID	References	Phytosociological association
ST1	Brullo e Marcenò 1984 Tab. 9 ril. 1-7	*Quercetum leptobalanae*
ST2	Brullo e Marcenò 1984 Tab. 9 ril. 17-18	*Oleo sylvestris-Quercetum virgilianae*
ST3	Brullo e Marcenò 1984 Tab 14 ril. 1, 2, 3, 6, 7	*Festuco heterophyllae-Quercetum congestae*
ST4	Brullo e Marcenò 1984 Tab. 12 ril. 1-5	*Arabido turritae-Quercetum congestae*
ST5	Brullo e Marcenò 1984 Tab. 3 ril. 9-12	*Oleo sylvestris-Quercetum virgilianae*
ST6	Brullo e Marcenò 1984 Tab. 3 ril. 9-12	*Oleo sylvestris-Quercetum virgilianae*
ST7	Brullo et al. 2001 Tab. 5 ril. 1, 3, 6, 7, 13	*Erico arboreae-Quercetum virgilianae*
ST8	Brullo et al. 2001 tab 23 ril. 1-4	*Erico arboreae-Quercetum congestae*
ST9	Brullo et al. 2001 tab 23 ril. 1, 4, 5 -14	*Erico arboreae-Quercetum virgilianae*
ST10	Brullo et al. 2001 tab 23 ril. 1-4	*Erico arboreae-Quercetum congestae*
ST11	Brullo e Marcenò 1984 Tab. 9 ril. 8-17	*Quercetum leptobalanae*
ST12	Brullo e Marcenò 1984 Tab. 11 ril. 1-8	*Erico arboreae-Quercetum virgilianae*
ST13	Brullo e Marcenò 1985 Tab. 14 ril. 4-5	*Festuco heterophyllae-Quercetum congestae*

In total, 3887 leaves and 1047 fruits were collected from 391 tree specimens. Since not all sampled tree specimens bore fruits, the final and complete matrix subjected to statistical analysis was composed of 333 specimens.

Table 4 Description of the morphological leaf characters analysed.

Variable	Code	Variable description
Area	Area	Leaf area (cm^2)
Perimeter	Per	Leaf perimeter (cm)
Petiole length	PL	Leaf petiole length from the base of the lamina to the base of the petiole (cm)
Lamina length	LL	Leaf lamina length (cm)
Lobe width	LW	Width of the major lobe, from the apex of the lobe to the central axis coinciding with the main vein of the leaf (cm)
Sinus width	SW	Distance between the maximum point of curvature of the sinus immediately below the main lobe and from the apex of the lobe to the central axis coinciding with the main vein of the leaf (cm)
Widest point	WP	Distance between the apex of the major lobe and the base of the lamina (cm)
Number of lobes	NL	Number of lobes on the left side of the leaf lamina, excluding the terminal central apex of the leaf lamina
Basal shape of the lamina	BSL	Basal shape of the lamina assessed on the left side on a scale varying from 1 to 9 (Kremer, 2002)
Compactness	Co	sqrt((4/pi)*area)/major axis
Obversity	Ob	WP/LL*100 (Kremer, 2002)
Petiole ratio	PR	PL/(LL+PL)*100 (Kremer, 2002)
Lobe depth ratio	LDR	(LW-SW)/LW*100 (Kremer, 2002)
Lobe width ratio	LWR	LW/LL*100 (Kremer, 2002)
Abaxial laminar pubescence	AB-PU	Assessed on voucher specimens using a scale varying from 0 to 6 (Kissling, 1980)
Adaxial laminar pubescence	AD-PU	
Twig pubescence	TW-PU	

Owing to the dichotomous keys of Flora D'Italia (Pignatti et al., 2017-2019) take into consideration the appearance of the bark ribs, all the sampled oak individuals were observed according to this aspect and photographed. As an example, in the supplementary files, the images (at three different levels of detail) relating to the trunks of the tree specimens collected in ST01 have been reported (Online Resource 1).

Table 5 Description of the morphological fruit characters analysed.

Variable	Code	Variable description
Cupula lenght	CL	Cupula length (mm)
Cupula width	CW	Cupula width (mm)

Acorn length	AL	Acorn length (mm)
Acorn width	AW	Acorn width (mm)
Cupula form	CW/CL	Ratio between cupula width and lenght
Cupula cover	CL/AL	Ratio between cupula and acorn lenght
Regularity of cupula edge	RCE	1= curved edge; 2= regular edge; 3= irregular edge
Scale shape	SS	1= triangular; 2= pyriform; 3= pyriform with gibbosity
Gibbosity	G	1= present; 2= absent
Cupula internal hairness	CIH	1= absent; 2= poor; 3= medium; 4= abundant
Type of cupula edge	TCE	1= scales discrete; 2= scales slightly protruding; 3= scales prying

Data analysis

The partition of the total variance of the leaf morphology was evaluated through the nested analysis of variance (nested ANOVA) on three hierarchical levels: a) inter-population (variance among different populations), b) intra-population (variance among the trees of a single population and c) individual (variance among the leaves of a single tree). A spreadsheet suitable for the purpose (McDonald, 2009) was modified to analyse more than 50 subgroups with up to 200 observations per subgroup and subsequently used to perform the two-level nested ANOVA (with Satterthwaite approximation, due to the unequal sample sizes of the subgroups) on the entire data set of 3887 leaves and 14 leaf variables.

The matrix of 333 individuals and 28 leaf and fruit variables was analysed using Syntax 2000 (Podani, 2001) and PAST 3.25 (Hammer et al., 2001). A cluster analysis was performed using the chord distance and the “minimum variance in new clusters” agglomerative method in order to identify possible diversity groups. Subsequently, a PCA ordination was performed using the correlation matrix.

For each group obtained from the cluster analysis, the mean and the standard deviation for morphological characters were calculated. Single box plots for each of the leaf and fruit morphological characters were also carried out. The consistency of the groups identified by the cluster analysis was tested by the Kruskal-Wallis test and multiple pairwise comparisons using Dunn's procedure (XLSTAT ver. 2019.2.2, ADDINSOFT, 1995-2019) for the categorical variables and by one-way ANOVA and Tukey-Kramer test (McDonald, 2009) for the numerical variables.

In order to validate the a-priori classification based on the guide species of the oak communities investigated, an expert classification of all the tree specimens collected was also performed based on the analytical key for oak identification recently published in the new edition of Flora d’Italia (Pignatti et al., 2017-2019). In some cases, our direct and expert use of this key made it necessary to do some forcing to complete the identification process of particularly critical specimens. When the incompatibility among diagnostic characters turned out to be too deep and would have required an excessive degree of subjectivity to complete the identification process of certain specimens, the latter were classified as “preliminarily indeterminate”. When we found a lower degree of incompatibility among diagnostic characters, it was decided to arbitrarily choose one of the two options proposed by the key of identification for completing the identification process based on our personal floristic and phytosociological experience. Specimens identified in this way were subsequently classified adding the epithet "doubtful" (e.g. *Q. virgiliana* doubtful, *Q. congesta* doubtful, etc.).

Our expert identifications of the specimens were compared with what was expected for the sampling sites based on the phytosociological tables of the associations already published for those areas or for strictly neighbouring areas. All the pubescent-oaks occurring at each collection site were collected by randomised sampling and not selectively directed toward the collection of one or the other pubescent-oak putative species. In relation to this, the expected values of the various oak species were obtained by making a correlation between the number of specimens we have collected and the values of frequency and coverage of the oak species reported in the phytosociological tables of the associations published for those areas.

Results

The nested ANOVA showed that the morphological character variability observed among the leaves of an individual tree (ranging between 42.40% and 89.73%) accounted for the highest portion of the total variability. This was followed by the variability observed among the leaves of the tree specimens belonging to the same population (9.37-46.73%) and by that observed among the leaves of the specimens belonging to different populations (0.83-17.72%) (Online Resource 2).

The cluster analysis of the matrix based on leaf and fruit characters (Fig. 2) produced a dendrogram showing the occurrence of two main groups of tree specimens (1 and 2), each of which divided into two further sub-clusters (Gr.: 1a, 1b, 2a, 2b). Both clusters and sub-clusters exhibited a rather low degree of reciprocal dissimilarity (< 0.022).

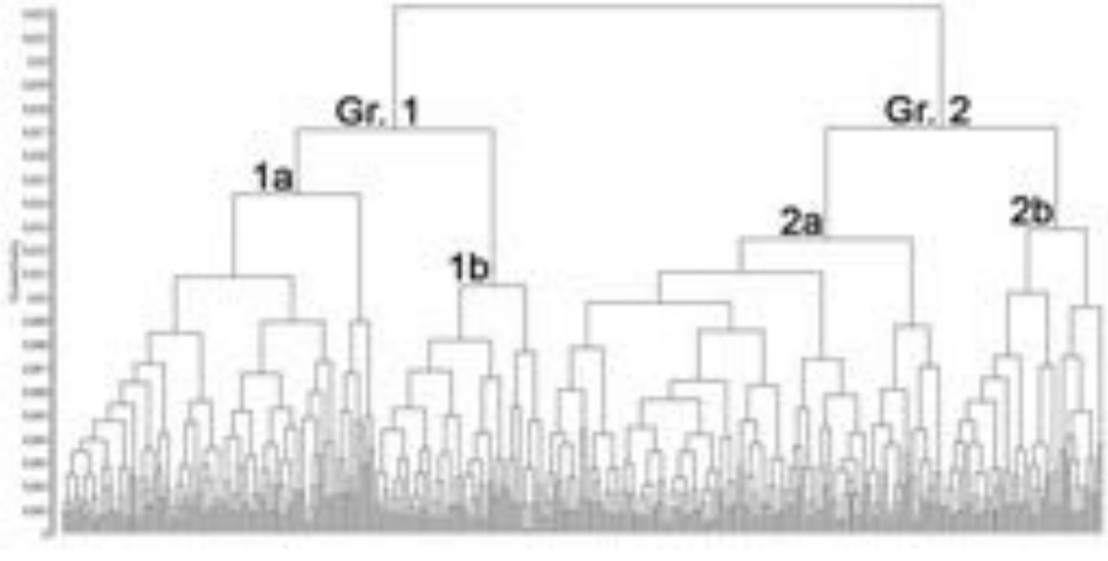


Fig. 2 Cluster analysis based on the 28 morphological traits.

The scarce degree of dissimilarity among the four groups identified by the cluster analysis is also confirmed and observable in the PCA diagram with partition superimposed, where the clusters were found to be significantly overlapping (Fig. 3). The variables correlated to the first PCA axis are the leaf dimensional ones (Area, Per, LL, LW, WP), while the variables correlated to the second PCA axis are the fruit dimensional ones (CW, AL, AW).

Ten oak populations out of the thirteen investigated were found to be composed of individuals that are distributed in all the four dendrogram clusters; two populations had individuals distributed in three clusters, and one population had individuals distributed in two clusters (Fig. 4).

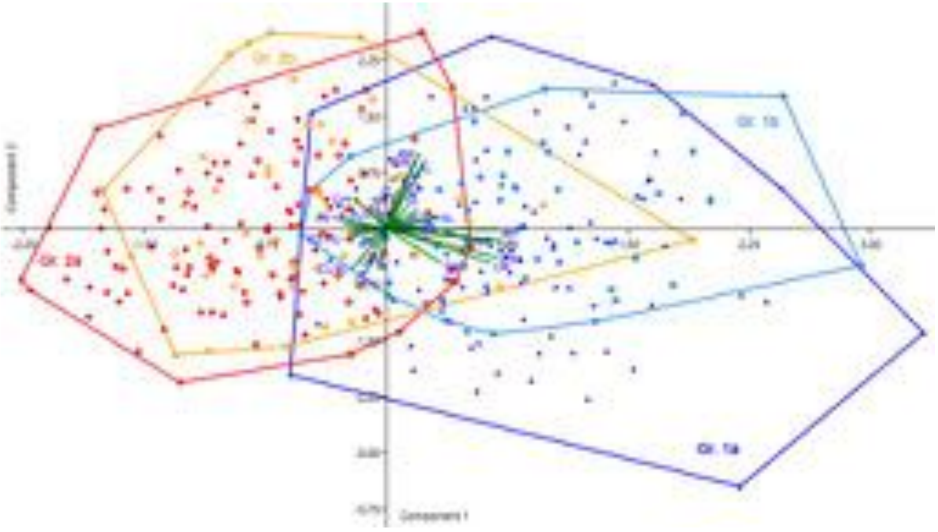


Fig. 3 Principal component analysis (PCA) based on the 28 morphological traits. The first axis bears 20.03% of the total variance while the second

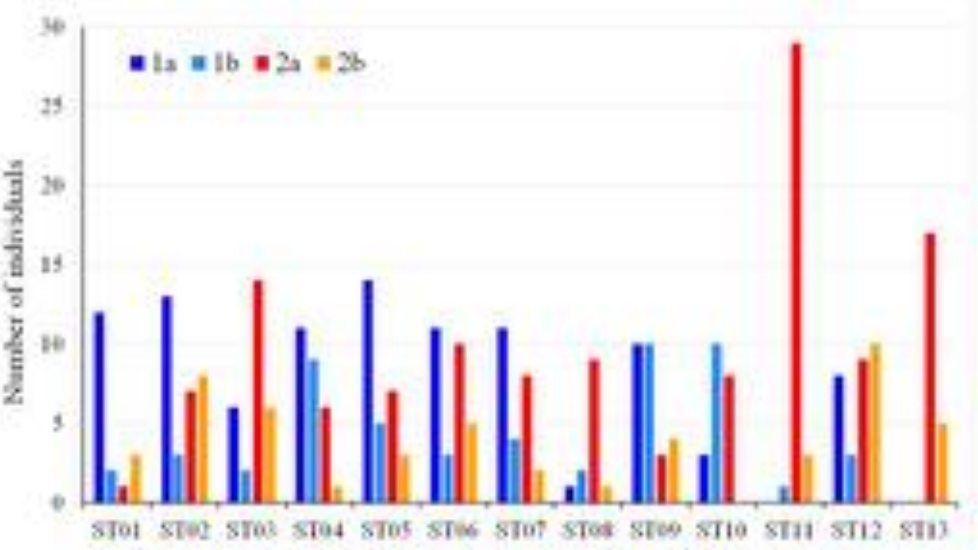


Fig. 4 Individual distributions of the thirteen populations inside the cluster analysis groups obtained.

Population ST11 (*Quercetum leptobalanae* of Piano Zucchi in the Madonie Mountains) was found to be the only one showing a large majority of tree specimens (88%) occurring in a single cluster (cluster 2a). It was followed by Population ST13 (*Festuco heterophyllae-Quercetum congestae,* Valle del Flascio in the Nebrodi Range), with 77% of tree specimens occurring in cluster "2a" and by Population ST01 (*Quercetum leptobalanae* Bosco di Ficuzza in the Busambra Massif) with 67% of its tree specimens occurring in cluster "1a". All the other oak populations investigated showed a less than 50% occurrence of their tree specimens in a single cluster.

When observing the box-plot diagrams, it was found that the separation of the two main clusters of the dendrogram (group 1 vs. group 2) was related to the following leaf dimensional characters: A, Per, LL, LW and WP (Online Resource 3). Two other characters, WP and LDR, were found to be involved in a further partition, which regarded the two main clusters separately (group 1a vs group 1b and group 2a vs. group 2b). The characters Co and Ob were found to discriminate inside cluster 1 (group 1a vs group 1b), while PL, LWR, NL, CW, AL, AW and CL/AL discriminated within cluster 2 (group 2a vs. group 2b). The one-way ANOVA partially confirmed these results (Table 6 and Online Resource 4).

Table 6 One-way ANOVA results for all the morphological characters considered in the four groups identified by cluster analysis. Different letters in the same column indicate significant differences among groups at $p < 0.01$ (Tukey-Kramer test).

Group	A	Per	PL	LL	LW	SW	WP	Co	Ob	PR
1a	30.143b	32.124b	1.318a	7.481c	2.585c	1.246b	4.32ab	0.761b	53.873b	14.129a
1b	29.085b	32.406b	1.300a	7.539c	2.579c	1.228b	4.178ab	0.737a	51.126a	14.39a
2a	18.111a	31.288b	1.235a	6.934b	2.386b	1.035a	7.025c	0.764b	53.348b	16.222b
2b	19.823a	26.71a	1.229a	6.395a	2.129a	1.026a	3.523a	0.764b	53.906b	16.005b
p-value	< 0.0001	< 0.0001	0.0669	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.005	< 0.005	< 0.0001

Group	LDR	LWR	NL	BSL	CL	CW	AL	AW	CW/CL	CL/AL
1a	49.812b	34.162ab	6.061bc	5.104ab	9.108a	12.816a	17.047bc	10.563b	1.453a	0.572a
1b	65.195d	34.076ab	6.060bc	5.308b	9.119a	12.868a	17.086bc	10.415ab	1.432a	0.563a
2a	55.082c	35.176b	5.413a	4.566a	8.857a	12.246a	14.806a	9.662a	1.417a	0.644b
2b	39.637a	33.269a	5.931b	4.759a	9.19a	13.014a	16.822b	10.222ab	1.445a	0.588ab
p-value	< 0.0001	< 0.01	< 0.0001	< 0.0001	0.6651	0.0893	< 0.001	< 0.01	0.7752	< 0.01

The Kruskall-Wallis test showed that the categorical characters CIH, AB-PU, AD-PU and TW-PU were diagnostic in separating sub-clusters 1a and 2a (Table 7 and Online Resource 5).

Table 7 Kruskal-Wallis test results, p-value for each cluster analysis group obtained. Different letters in the same column indicate significant differences among groups at $p < 0.05$ (multiple pairwise comparisons using Dunn's procedure).

Group	RCE	SS	G	CIH	TCE	AB-PU	AD-PU	TW-PU
1a	a	a	a	a	a	a	a	a
1b	a	a	a	ab	a	ab	ab	ab
2a	a	a	a	b	a	b	b	b

2b	a	a	a	ab	a	ab	ab	ab
p-value (one-tailed)	*0.482*	*0.503*	*0.382*	*0.021*	*0.449*	*< 0.0001*	*< 0.0001*	*0.003*

The expert identification of the 333 samples carried out through the rigorous use of the analytical keys of the most recent edition of Flora d'Italia (Pignatti et al. 2017-2019) showed a result different from that proposed by the cluster analysis. Regarding this, it must be said that it was not possible to complete the identification process for all the 333 tree specimens since 30 of them were classified as preliminary/indeterminate and 86 as doubtful. The only taxon identified without incurring doubtful specimens was *Q. ichnusae*.

The comparison between the number of specimens identified and that of the specimens expected to be found based on the already published phytosociological papers (Table 8) showed the following figures. Both for *Q. congesta* (36/82 including doubtful specimens) and for *Q. dalechampii* (88/102 including doubtful specimens) a number of specimens were identified that are not too far from the number of expected specimens (64 and 93 specimens respectively). More than half (46) of the doubtful tree specimens were identified as *Q. congesta,* which is the only taxon, among those investigated, that showed a percentage of doubtful specimens higher than that of the identified specimens.

More than half (19) out of the 36 specimens identified as *Q. congesta* came from sites were no specimens of *Q. congesta* were expected. The percentage of unexpected *Q. dalechampii* specimens (21) in the total of those identified (88) was significantly smaller than that of *Q. congesta*.

However, in two of the three sites from which these unexpected specimens came from (all referring to the association *Oleo-Quercetum virgilianae*), *Q. dalechampii* represented the most abundant species, where instead, from phytosociological literature data, it should be absent. The specimens identified as *Q. virgiliana* were found to be 12 (17, adding 5 doubtful *Q. virgiliana* specimens), whereas the number was expected to be 110. These expectations were because three sites had been sampled (totalling 85 specimens) where, from literature, they should have corresponded to pure *Q. virgiliana* woods or, at most, mixed with scattered individuals of *Q. amplifolia*. The total number of specimens attributable to *Q. leptobalana* was found to be 39 (51, adding 5 doubtful *Q. leptobalana* specimens). Only 14 specimens (36%) out of the 39 *Q. leptobalana* specimens identified were collected in the two sites reported as *Quercetum leptobalanae* (4 specimens in ST1 and 10 in ST11), while the remaining specimens were collected in other sites in both Sicily and southern Calabria. No samples were found to be classifiable as *Q. amplifolia*. We also identified individuals of *Q. pubescens* (35) and *Q. ichnusae* (7) when no individuals were expected, as these two oak taxa were not considered to present in the study area in Pignatti et al. (2017-2019).

Discussion

In the last thirty years, a major international effort has been made to revise the entire *Quercus* genus that had reached the number of 500-650 species worldwide (Goavert and Frodin, 1998). Several oak "putative" taxa were progressively eliminated following the rules of the Angiosperm Phylogeny Group (2009) and the International Code of Nomenclature considering invalid names, synonymies and publication priorities, among others. The most recent reference taking into account the genus *Quercus* as a whole lists 435 species (Denk et al., 2017). In southern Europe, unfortunately, the taxonomical framework of the oak taxa at the species level is not yet well defined, especially within the *Quercus pubescens* collective group that remains a still-unresolved tangle. The papers published in the last fifteen years on the morphological variability of the European white oaks, both in the Italian Peninsula and in south-eastern Europe, are all moving in the same direction, that is to critically and statistically evaluate the real consistence of numerous oak taxa regardless of whether or not they were included in the national floras or checklists as accepted species (Franjić et al., 2006; Enescu et al.,2013; Wellstein and Spada, 2015; Di Pietro et al., 2016; Di Pietro et al., in press; Fortini et al., 2019).

What is emerging from this accurate review of general work is that the taxonomic investigation within white oaks based on morphological characters (micro and macro) allows, at most, discriminating in between already well-identifiable species. In the specific case of southeastern-European white oaks, this means being able to identify with some certainty the following species: *Q. robur, Q. frainetto, Q. petraea* and *Q. pubescens*. As regards the first three taxa, there have never been serious doubts about the fact that they had to be accepted as valid species and taxonomically distinct from each other, and only a few doubts concerning the fact that any morphological variations within them had to be taxonomically translated at the rank of variety, or at most, subspecies (see *Q. petraea* subsp. *austrotyrrhena* or *Q. robur* subsp. *brutia*). Only within the collective group of *Q. petraea* is there currently some nomenclatural movement due to the reorganisation of the group following the shift of the name *Q. dalechampii* into the collective group of *Q. pubescens* (cf. Di Pietro et al., 2012; Raab-Straube and Raus, 2013; Kucera, 2017). This is nothing comparable, however, with the taxonomic and nomenclatural chaos in which the group of *Q. pubescens* is immersed, where the wide morphological variability affecting this group has often been used to advance proposals to split it at the rank of species.

Italy is certainly among the southern European countries where the divisionist tendency is more deeply rooted. According to the detractors of the "minimalist" front (the latter being composed of those who tended to reduce the number of white oaks of the Italian peninsula to four species only), there would be numerous reasons that lead the “minimalists” to (wrongly) underestimate the real number of pubescent-oaks species. One of the most popular criticisms (never clearly expressed) regards the fact that the collectors would not always be aware of what they would be collecting. Other criticisms are in regards to a) the lack of knowledge about the “real” diagnostic characters to be observed in the specimens collected, which consequently lead to not using the right ones in the identification stage, b) the scarce geographical or ecological knowledge of the sampling area that leads to selecting “wrong” collection sites not suitable to host the most typical populations of the species in issue and c) the custom of collecting too few specimens within an oak community with a low possibility that the individuals of the species in issue would end up among those collected.

Whether it was for one or another reason, or all of them together, the final result is that some authors continued to refer to names of pubescent-oak taxa different from *Q. pubescens* in their floristic, taxonomic and phytosociological contributions and, using these names, to describe associations, such as *Quercetum leptobalanae, Oleo-Quercetum virgilianae, Festuco-Quercetum congestae,* or even higher rank syntaxa, such as *Quercenion virgilianae, Quercenion dalechampii, Pino-Quercion congestae* and *Quercetalia virgilianae* (cf. Brullo, 1984; Brullo and Marcenò, 1985; Brullo et al., 1999; Brullo et al., 2001; Blasi et al., 2004; Brullo et al., 2009).

In our opinion, the problem is partly conceptual and partly methodological. In broad terms, the ascertained wide morphological variability existing not only within the whole pubescent-oaks group but also within the same population of the same putative pubescent-oak species is not considered a deterrent factor on the possibility of operating an expansive taxonomic policy.

On the contrary, the opposite is considered true; it is unlikely that such a wide variability could be addressed to a single species. This position is generally maintained regardless the evidence that when making collections that include a large number populations and a large number of tree specimens per population, this variability follows an about continuous and gradual pattern of variation for nearly all the diagnostic morphological characters. In Di Pietro et al. (2016), twenty-four populations of pubescent oaks were morphologically and molecularly investigated in the Apulian Peninsula. Great attention was paid in covering the entire bioclimatic and lithological variability of the Apulia region, in choosing forest sites for which there were phytosociological tables already published and clearly indicating the guide oak species and in collecting an adequate number of tree individuals within populations. After submitting the dataset to multivariate analysis and biometric measurements, the final result was that no groups of morphological and molecular diversity and no combination of diagnostic characters could be significant in identifying Apulian pubescent oaks. There was nothing unusual about this, except that the samplings were carried out in communities originally described as dominated by different oak species such as *Q. virgiliana, Q. dalechampii* and *Q. pubescens* (Biondi et al., 2004; Di Pietro and Misano, 2009). Also, the fractal analysis applied to the leaves of Calabrian deciduous oaks (Musarella et al., 2018) does not distinguish the species of *Q. pubescens* group.

In the present research on the Calabrian and Sicilian oaks, we decided to further improve the sampling protocol by increasing the number of individuals per station and to include, among the sampling sites, the '*loci classici'* of some of the species accepted as present within the most important Italian flora checklists (e.g. Pignatti, 1982; Brullo et al., 1999; Pignatti et al., 2017-2019; Bartolucci et al., 2018). The result was inevitably the same; no diversity groups were found at the morphological level, whereas, according to the published phytosociological studies, we should have found the presence of *Q. virgiliana, Q. congesta, Q. amplifolia, Q. dalechampii* and *Q. leptobalana*. The high frequency and cover values of these species in the original phytosociological tables were such that it would have been highly unlikely not to collect any sample of one or more of these species based on the randomised sampling protocol adopted. The cluster analysis output showed four major groups. We have been open in considering the cluster analysis result as possibly reliable, even if the degree of dissimilarity of the four groups highlighted was below an acceptable statistical threshold. Such a result, not too distant from what was expected (four groups for five putative species), would still have been acceptable if these four groups had shown some signs of homogeneity, either morphological, ecological or geographical. On the contrary, the distribution of individuals in the four groups was found to be random and seemingly related to none of the aforementioned criteria.

Each of the four groups identified by the cluster analysis was found to be composed of individuals coming from all the 13 sites of collections investigated. For example, individuals collected in a thermo-Mediterranean limestone site (Marineo) for which the phytosociological literature reported a xero-thermic *Q. virgiliana-Q. amplifolia* community were found to segregate in association with individuals from the montane belt of the Etna Volcano where a *Q. congesta-Q. dalechampii* association (with a total absence of *Q. virgiliana* and *Q. amplifolia*) was reported in the literature.

Such a surprising result is difficult to explain, especially if our intent was that of attributing a taxonomical meaning to the cluster analysis.

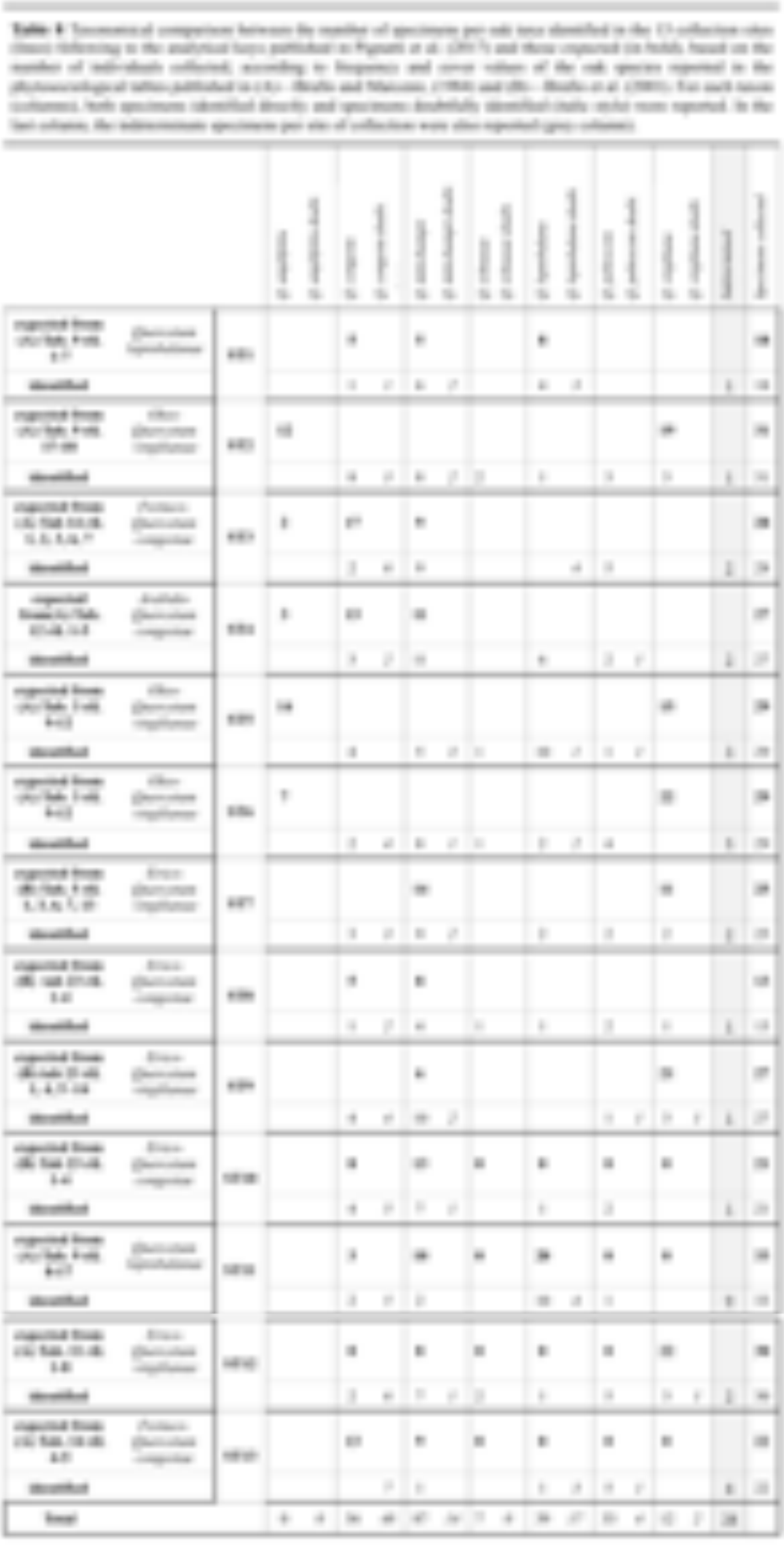
It is also difficult to explain the fact that most of the individuals from the site "Piano Zucchi" (located in the Madonie mountains and *locus classicus* of the *Quercetum leptobalanae*) clustered in group 2a, while those of the *Quercetum leptobalanae* from the site "Bosco Ficuzza" clustered in group 1a (Fig. 4). Obviously, this result is even more surprising if we take for granted, as it is logical to suppose, that in *Quercetum leptobalanae,* the physiognomic dominance should be given by *Q. leptobalana* and the other oak species (if present) would necessarily have to show lower frequency and coverage values. On the basis of the phytosociological tables published for these two sites (Brullo and Marcenò, 1984)*, Quercetum lepobalanae* included four species of deciduous oaks, each of which showed variable frequency and cover indices, and *Q. leptobalana* was undoubted to be considered the dominant species. For this reason, it sounds strange that the individuals collected in these two *Quercetum leptobalanos* sites do not occur, for the most part, in the same dendrogram cluster. It is also strange that a considerable percentage of individuals coming from sites where only *Q. virgiliana* and *Q. amplifolia* occur ended up in groups 1a and 2a, which are where most individuals of *Quercetum leptobalanae* are concentrated (notably, *Q. virgiliana* was not reported in any of the two original phytosociological tables of *Quercetum leptobalanae*).

The characters that in our investigation were found to be, at least in part, diagnostic for the four clusters of the dendrogram groups delineate four morphotypes. Unfortunately, these morphotypes do not seem to match any of the putative oak species that were the object of this study, neither referring to their original descriptions nor those recently proposed in analytical keys (Brullo et al., 1999; Pignatti et al., 2017-2019). Many morphological characters that are currently considered diagnostic, especially dimensional characters (e.g. leaf and acorn length) are known to be very dependent on the seasonal variations of the climate factors or stand conditions (cfr. Skvork et al., 2005; Bonito et al., 2011; Martiník et al., 2014). For this reason, they can exhibit a wide range of variation, from one year to another, and lead to misinterpretations when used in diagnostic keys for identification purposes.

In theory, we could venture the hypothesis that the use of the complete set of morphological characters in statistical processing, even if correct from a methodological point of view, could determine an excessive background noise linked to the negative incidence of non-diagnostic characters and result therefore in a chaotic mixture of individuals released from the taxonomic reality in both the dendrogram and PCA diagram. This possibility is however largely refuted by the recent taxonomic literature, which, at least in the first stages of the statistical analysis, tends to use the greatest number of characters to discriminate at the interspecific and intraspecific levels. Moreover, even when only considering the recent taxonomic literature concerning the southern Italian white oaks (Viscosi and Fortini, 2011; Fortini et al., 2015), it has been shown that if populations of other white oak species (*sensu* Nixon, 1993), such as *Q. petraea* and *Q. frainetto*, were included in statistical analysis procedures together with populations of *Q. pubescens* s.l., the specimens belonging to *Q. petraea* and *Q. frainetto* tended to separate quite clearly from the undifferentiated bulk of specimens belonging to *Q. pubescens* s.l.

Aware of the difficulty in identifying some of the white oak taxa, we have also tried other ways to identify and interpret the specimens collected in this research. Among these, there was that of not taking into account the objective results of the statistical analysis of the data-set composed of the morphological characters of leaves and fruits, but to perform subjective identification using the analytical keys proposed in Brullo et al. (1999) and further updated in Pignatti et al. (2017-2019). It must be said, however, that even the direct and expert use of the analytical keys for the identification of the species has represented a tortuous path that in some cases—as explained in the data and methods paragraph—led us to do some forcing to complete the identification process. This was made obvious when, for about 30 tree individuals out of 333 sampled, we found it impossible to assign them to one or the other pubescent-oak putative species, so we decided to classify them as '*preliminarily indeterminate'*. In these cases, we found that where some diagnostic characters addressed the identification choice towards a given oak taxon, some others were inconsistent with that choice. For example, in the Madonie Mountains (Sicily) we found specimens that were compatible with *Q. leptobalana* when considered for acorn size and with *Q. dalechampii* when considered for leaf shape. In the Palermo Mountains, we found specimens for which the bark features led univocally to *Q. dalechampii*, whereas the cupule and acorn traits led towards *Q. virgiliana* or *Q. congesta*.

It is interesting to note, that the expert identification showed a result sharply different from not only the result of the cluster analysis but also from what was expected based on the phytosociological tables already published for the same forests sampled by us or for oak forests existing in similar environmental conditions and located in their vicinity. Actually, we would have expected a numerical division of specimens pertinent with the bioclimatic and ecological characteristics of the sites of collection or, at least, with the specific composition of the dominant tree layer of the sampled communities. These expectations were largely unmet. The samples attributable with certainty to *Quercus virgiliana* are only 12, whereas we expected not less than 100 individuals to be assigned to it. This expectation was not really so improbable given that among the sampling sites there were three of these which, from literature, were considered as pure *Q. virgiliana* woods (at most with some individuals of *Q. amplifolia*). The direct attribution of the tree specimens to *Q. leptobalana* should have been among the simplest because the dimension of cupule and acorn are described to be the smallest among those of all the putative oak species considered in this work. Moreover, the *Quercetum leptobalanae* is reported in the literature for two Sicilian sites only (cf. Brullo, 1984; Brullo and Marcenò, 1985; Gianguzzi and La Mantia, 2004) and we sampled those sites. The total specimens attributable to *Q. leptobalana* were 39, a reasonable number that would be approximately compatible with the two samplings of 35 individuals each in the two sites of *Quercetum leptobalanae* (ST1 and ST11). However, by analysing the data reported in Table 8**,** it turns out that only 14 specimens identified as *Q. leptobalana* (36%) fell into the two sites in question (4 specimens in ST1 and 10 in ST11), while the remaining specimens were redistributed in other collection sites. Among these, the "Ceraulo" site of collection, which was located at the Etna Volcano footslope, hosts as many as 10 specimens of *Q. leptobalana* despite this taxon never being reported for the Etna region or, more generally, for eastern Sicily. Obviously, our results could be new records for *Q. leptobalana* in Sicily, and nothing would forbid the three specimens we also identified as *Q. leptobalana* from the Calabrian sites from being considered as the first records of this taxon for continental Europe. Conversely, no specimens were found to be classifiable as *Q. amplifolia,* although, based on the phytosociological literature, this species should occur in at least 60% of the communities we sampled. Another result, which is hardly explainable in light of what is reported in the most recent diagnostic keys, is our identification of individuals attributable to *Q. pubescens* (35) and *Q. ichnusae* (7), because the first species is considered absent in southern Italy and the second is considered endemic to Sardinia Island (Mossa et al., 1999.



In truth, what the taxonomists are slowly becoming aware of is that the current stage of identification of the pubescent-oak specimens does not allow univocally identifying them at the species rank until the collective group of *Q. pubescens* s.l. is reached. Once one gets to the objective dichotomy between the "pubescent-twig oaks" (*Q. pubescens* collective group) and the "glabrous-twig oaks" (*Q. petraea* collective group) the subsequent steps in taxonomic discrimination become markedly subjective (for both groups) and therefore not universally shared. As regards Italy, we are quite sure about the identification at the rank of species of the four main entities of white oaks, namely *Q. pubescens, Q. frainetto, Q. petraea* and *Q. robur*. Alongside these, botanists often come across individuals with very variable phenotypic aspects and intermediate values of characters, such as leaf hairiness, humps of cupule scales, depths of leaf sinuses and lobe numbers, among others. This phenomenon is perfectly explainable by the natural tendency of oaks, and among the white oaks especially of *Q. pubescens* s.l., to be abundantly subject to hybridisation and introgression.

It is possible that in southern Italy and Sicily the morphological variability of the group of pubescent-oaks could be objectively higher than that occurring in other countries, and this fact may have legitimately authorised ranks of botanists, from the early 19th century (Presl & Presl, 1926; Tenore, 1930, 1935-36; Gussone, 1944) to nowadays (Brullo et al., 1999; Giardina et al., 2007; Pignatti et al., 2017-2019) to hypothesise a multi-specific collective group. As far as we are concerned, we are more likely to think that this high morphological variability (if it is ascertained that it is actually higher than that observable in similar populations of other areas) has its logical explanation in the fact that southern Italy has represented one of the most important refuge sites for the thermophilous forest during the Quaternary cold periods. Accordingly, southern Italy oak species have probably had higher possibilities than the oak species living in northern areas to be in close contact for very long periods and possibly to produce hybrids. At this point, the question would arise spontaneously about which oaks would hybridise. Our opinion is that these would be hybrids among those species of white oaks for which we would still able to recognise the parentals, such as *Q. pubescens, Q. petraea, Q. robur* and *Q. frainetto*. However, by adopting a more markedly panmittic view, one could come to think that the parentals were all members of the collective group of *Q. pubescens*, and therefore give reason to those botanists who consider this collective group composed of numerous other species in addition to *Q. pubescens* s.s. At present, we are not yet able to give definitive answers on this point. However, support for the first hypothesis comes from other studies already published for southern Italy (Di Pietro et al., 2015) where the presence of the genomes of *Q. frainetto, Q. petraea* and *Q. pubescens* were identified (in variable quantities) in several oak individuals showing intermediate morphological characters, collected in mixed-woods where the aforementioned oak taxa played the role of guide-species.

As already pointed out in other works concerning oak taxonomy, it is not always possible to establish the status of accepted species basing only on morphometric studies. Such studies cannot serve, especially when the description of new species or the identification of a specimen is based on the observation of a low number of specimens (with the extreme case of the type specimens only) and collected from a single population. Even in studies that cover large geographical areas and that compare populations coming from sites very distant from each other, there is the tendency to collect a low number of individuals per population. Therefore, while on the one hand results of global interest are produced, especially in the phylo and phytogeographical keys, on the other hand, there is the risk of considering a very limited part of the variability existing at local or regional level as sufficiently diagnostic for taxonomical interpretation. Studies which are planned to cover uniformly and in more detail smaller areas and to analyse a high number of specimens per populations are perhaps less successful in terms of publishing and audience but provide a more realistic picture of the range of morphological variability of a species.

All the results obtained in this research, suggest that the most plausible hypothesis is that, at least in the study area, all the populations collected refer to a single species of pubescent oak characterised by a high morphological variability and presumably ecological amplitude. A name that would respond perfectly to the identikit of this morphologically and ecologically highly variable species would actually be that of *Q. pubescens* Willd. This statement, despite being in contradiction with what is reported in the taxonomic and phytosociological literature (including the very recent one) concerning southern Italy, are actually in accordance with what has already been published for the whole of southern Europe about the impossibility of tracing reliable taxonomic boundaries within the morphological variability of the collective group of *Q. pubescens*.

Significantly more complete and consistent information useful to unravel the taxonomic-nomenclatural tangle concerning species-discriminating characters in oaks could be derived from comparative studies combining morphological and molecular analyses. Interesting results on the association between genomic region and leaf traits were recently obtained in a study on two inter-fertile and partially sympatric red oak species of north-America (Gailing et al., 2018). As regards the white oaks, the preliminary results of the genetic analysis carried out on the same set of populations and individuals used in this paper (Di Pietro et al., in prep.) are showing a significant concordance between molecular and morphological results. However, similar comparative studies have already been published for other areas of southeastern Italy (Fortini et al., 2015; Di Pietro et al., 2016, in press) and asserted that the occurrence of more than one species of pubescent oak, at least for those areas, was unlikely.

Conclusion

Sicily Island and southern Calabria form a very important phytogeographical district, which is located in the centre of the Mediterranean Basin and is notoriously populated by a high number of endemics, relics and rare species (Brullo et al., 2011; Sciandrello et al., 2015; Spampinato et al., 2018). As far as the genus *Quercus* is concerned, this area is known for hosting several pubescent-oak species, some of which (*Q. congesta, Q. amplifolia, Q. leptobalana* and *Q. dalechampii*) were typified precisely there and *Q. virgiliana* in another site of southern Italy. These species, in addition to being all accepted in the latest edition of the Flora of Italy (Pignatti et al., 2017) and, some of them, in *Flora Europaea* and in other National Floras and checklists, were used as guide species to describe a high number of phytosociological syntaxa. Furthermore, these oak taxa are currently considered as diagnostic species for some habitats of the 92/43/EEC Directive (Biondi et al., 2009; EC, 2013). In this study, a morphometric statistical analysis of leaf and fruit characters of 14 pubescent-oak populations from Sicily and southern Calabria was carried out in order to identify the occurrence of possible clusters of morphological diversity.

The statistical analysis of the specimens made without an *a-priori* identification and the parallel expert identification of the same specimens based on the use of analytical keys provided by the last edition of Flora of Italy (Pignatti et al., 2017-2019) showed results that were incompatible with each other and did not correspond to what is published to date concerning the number and distribution of taxa in the study area.

The results of multivariate analysis procedures showed a very low degree of dissimilarity among tree specimens and sampled populations, as well. Pubescent-oak specimens coming from populations that, according to the published literature, should not have shared oak taxa that were located in very different ecological situations were found to segregate in close association in the cluster analysis and PCA diagrams.

The morphological characters considered diagnostic in the identification keys of the main Italian and European Floras do not allow, at present and in the study area, the identification of groups such as to support five different species in the collective group of *Q. pubescens*. On the contrary it would seem that the morphological variability found refers to a single species.

The result obtained has an objective importance that goes beyond the boundaries of the study area and takes on European significance. In fact, if, as appears from the preliminary results, even the molecular data should confirm the morphological ones, it is probable that both the taxonomic schemes and the nomenclatural articulation of the genus *Quercus* will undergo a significant revision in the near future and the same will happen in a syntaxonomic key both in the Eurovegchecklist (Mucina et al., 2016) and in the Prodrome of the Italian vegetation (Biondi et al., 2014).

Considering the current stalemate in recognising a taxonomic diversity that struggles to emerge at the species level, it would be worthwhile to lower the taxonomic target a little and encourage other types of investigations. For example, intraspecific investigations aimed at re-establishing territorial mapping of pubescent oaks at the eco-typical level, capable of highlighting situations of both morpho-genetic isolation and wide distribution, may be useful for the knowledge of the systematics of *Quercus* as well as for the conservation of the diversity of the genus at all its levels.

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Information on Electronic Supplementary Material

Online Resource 1 Bark features of the oak individuals sampled at "Bosco Ficuzza" (ST01 **-** Palermo, Italy), 29-09-2017 (Photos Orazio Caldarella).

Online Resource 2. Two-level nested ANOVA results for the morphological leaf characters.

Online Resource 3. Box-plot showing the minimum, first quartile, median (line), mean (cross) third quartile, and maximum together with both limits beyond which values are considered anomalous (symbols ° and *) for all the morphological leaf and fruit characters in the four groups identified by cluster analysis.

Online Resource 4. One-way ANOVA complete results for all the morphological leaf and fruit characters in the four groups identified by cluster analysis.

Online Resource 5. Kruskal-Wallis results for the morphological leaf and fruit characters in the four groups identified by cluster analysis.

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SM3. A multivariate morphometric analysis of phytognostic traits in southern Italy and Sicily pubescent-oaks

Di Pietro R.[1], Conte A.L.[2], Di Marzio P.[2], Gianguzzi L.[3], Spampinato G.[4], Caldarella O.[3], Fortini P.[2].

[1]Department PDTA, University of Rome Sapienza, 00196 Rome, Italy.
[2]Department of Bioscience and Territory, University of Molise, 86090 Pesche (IS) Italy.
[3]Department of Scienze Agrarie e Forestali, University of Palermo, Italy.
[4]Department of AGRARIA, Mediterranean University of Reggio Calabria, Italy.

✉: Corresponding author, Romeo Di Pietro: romeo.dipietro@uniroma1.it

Online Resource 1 Bark features of the oak individuals sampled at “Bosco Ficuzza” (ST01 - Palermo, Italy), 29-09-2017 (Photos Orazio Caldarella).

ST01, Sample 1.

ST01, Sample 2.

ST01, Sample 3.

ST01, Sample 4.

ST01, Sample 6.

ST01, Sample 7.

ST01, Sample 9.

ST01, Sample 10.

ST01, Sample 11.

ST01, Sample 12.

ST01, Sample 13.

ST01, Sample 14.

ST01, Sample 15.

ST01, Sample 16.

ST01, Sample 17.

Station 1 Sample 18.

ST01, Sample 19.

ST01, Sample 20.

ST01, Sample 21.

ST01, Sample 22.

ST01, Sample 23.

ST01, Sample 24.

ST01, Sample 25.

ST01, Sample 26.

ST01, Sample 27.

ST01, Sample 28.

ST01, Sample 29.

ST01, Sample 30.

Online Resource 2 Two-level nested ANOVA results for the morphological leaf characters.

Area	**sum of squares**	**d.f.**	**mean square**	**Fs**	**P**	**variance component (%)**
among groups	80279.728	12	6689.977	15.679	0.000	17.72
subgroups within groups	161285.978	378	426.682	7.060	0.000	31.16
within subgroups	211299.000	3496	60.440			51.12
total	452864.707	3886				100.00

Per	**sum of squares**	**d.f.**	**mean square**	**Fs**	**P**	**variance component (%)**
among groups	59192.566	12	4932.714	12.307	0.000	16.91
subgroups within groups	151409.928	378	400.791	10.538	0.000	40.68
within subgroups	132878.717	3496	38.009			42.40
total	343481.212	3886				100.00

PL	**sum of squares**	**d.f.**	**mean square**	**Fs**	**P**	**variance component (%)**
among groups	30.496	12	2.541	3.587	0.000	4.44
subgroups within groups	267.684	378	0.709	10.515	0.000	46.73
within subgroups	235.452	3496	0.067			48.82
total	533.632	3886				100.00

LL	**sum of squares**	**d.f.**	**mean square**	**Fs**	**P**	**variance component (%)**
among groups	1925.953	12	160.496	17.842	0.000	20.92
subgroups within groups	3398.321	378	8.995	7.998	0.000	32.67
within subgroups	3929.801	3496	1.124			46.41
total	9254.074	3886				100.00

LW	**sum of squares**	**d.f.**	**mean square**	**Fs**	**P**	**variance component (%)**
among groups	234.681	12	19.557	14.459	0.000	15.43
subgroups within groups	511.006	378	1.353	6.146	0.000	28.85
within subgroups	768.945	3496	0.220			55.72
total	1514.632	3886				100.00

SW	sum of squares	d.f.	mean square	Fs	P	variance component (%)
among groups	66.638	12	5.553	8.968	0.000	10.32
subgroups within groups	233.941	378	0.619	6.862	0.000	33.27
within subgroups	315.301	3496	0.090			56.41
total	615.880	3886				100.00

WP	sum of squares	d.f.	mean square	Fs	P	variance component (%)
among groups	598.458	12	49.871	13.253	0.000	11.77
subgroups within groups	1421.689	378	3.763	4.350	0.000	22.24
within subgroups	3022.469	3496	0.865			65.99
total	5042.616	3886				100.00

Co	sum of squares	d.f.	mean square	Fs	P	variance component (%)
among groups	0.528	12	0.044	2.321	0.007	2.24
subgroups within groups	7.158	378	0.019	9.689	0.000	45.60
within subgroups	6.830	3495	0.002			52.16
total	14.515	3885				100.00

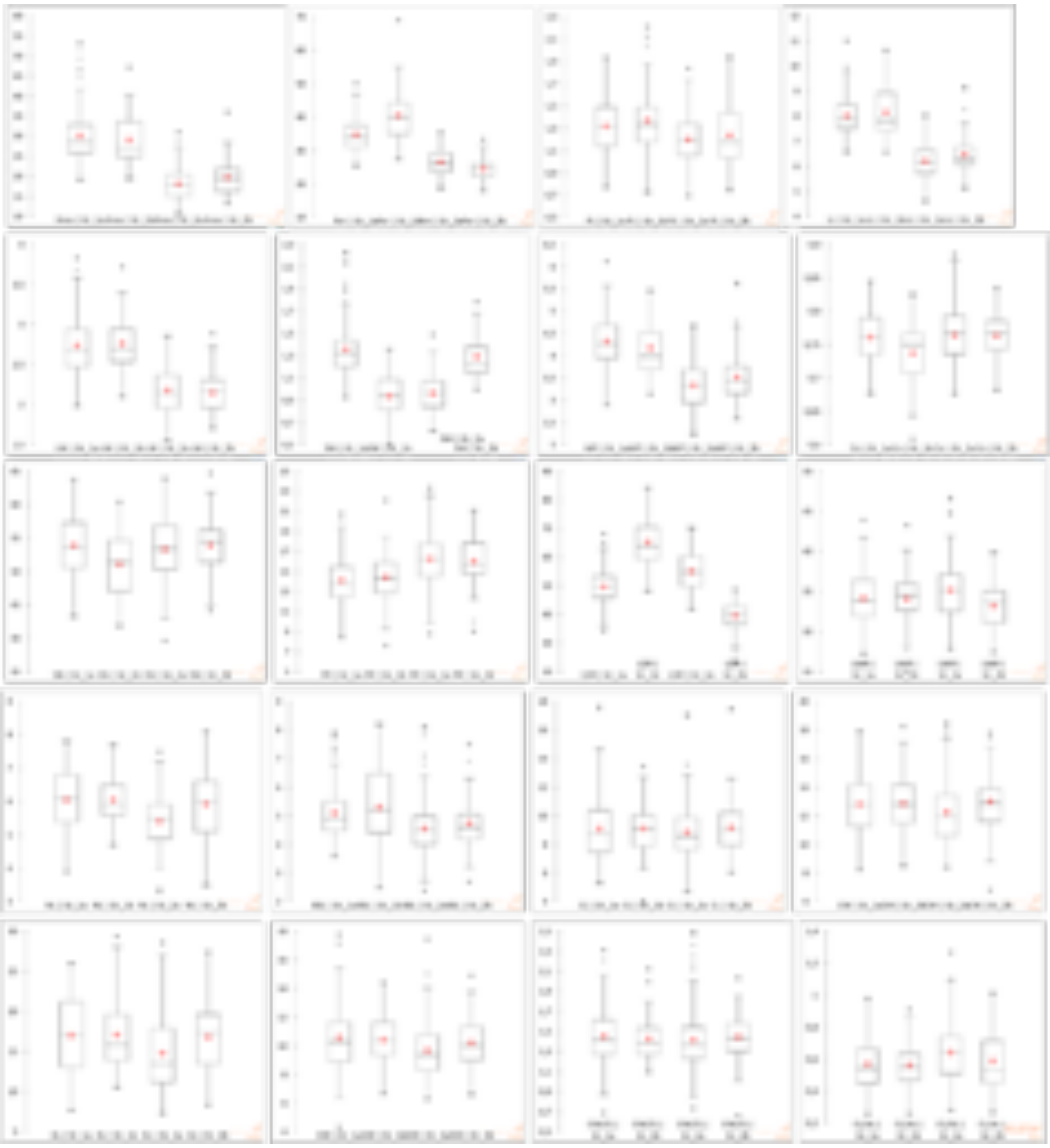
Ob	sum of squares	d.f.	mean square	Fs	P	variance component (%)
among groups	6337.642	12	528.137	2.468	0.004	0.90
subgroups within groups	80849.671	378	213.959	2.038	0.000	9.37
within subgroups	366882.983	3496	104.944			89.73
total	454070.296	3886				100.00

PR	sum of squares	d.f.	mean square	Fs	P	variance component (%)
among groups	4543.934	12	378.661	5.025	0.000	6.98
subgroups within groups	28465.188	378	75.349	11.372	0.000	47.50
within subgroups	23149.522	3496	6.622			45.52
total	56158.644	3886				100.00

LDR	sum of squares	d.f.	mean square	Fs	P	variance component (%)
among groups	53997.005	12	4499.750	5.305	0.000	6.20
subgroups within groups	320428.957	378	848.176	7.671	0.000	37.66
within subgroups	386352.622	3496	110.513			56.13
total	760778.584	3886				100.00

LWR	sum of squares	d.f.	mean square	Fs	P	variance component (%)
among groups	2372.373	12	197.698	1.588	0.093	0.83
subgroups within groups	47027.900	378	124.481	6.724	0.000	36.24
within subgroups	64683.448	3496	18.502			62.93
total	114083.720	3886				100.00

Online Resource 3 Box-plot showing the minimum, first quartile, median (line), mean (cross) third quartile, and maximum together with both limits beyond which values are considered anomalous (symbols ° and *) for the morphological leaf and fruit characters in the four groups identified by cluster analysis.



Online Resource 4 One-way ANOVA complete results for the morphological leaf and fruit characters in the four groups identified by cluster analysis.

[illegible]	SS	df	MS	Fs	p	[illegible]
among groups	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
within groups	[illegible]	[illegible]	[illegible]			[illegible]
total	[illegible]	[illegible]				

[illegible]	SS	df	MS	Fs	p	[illegible]
among groups	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
within groups	[illegible]	[illegible]	[illegible]			[illegible]
total	[illegible]	[illegible]				

[illegible]	SS	df	MS	Fs	p	[illegible]
among groups	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
within groups	[illegible]	[illegible]	[illegible]			[illegible]
total	[illegible]	[illegible]				

[illegible]	SS	df	MS	Fs	p	[illegible]
among groups	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
within groups	[illegible]	[illegible]	[illegible]			[illegible]
total	[illegible]	[illegible]				

[illegible]	SS	df	MS	Fs	p	[illegible]
among groups	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
within groups	[illegible]	[illegible]	[illegible]			[illegible]
total	[illegible]	[illegible]				

[illegible]	SS	df	MS	Fs	p	[illegible]
among groups	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
within groups	[illegible]	[illegible]	[illegible]			[illegible]
total	[illegible]	[illegible]				

[illegible]	SS	df	MS	Fs	p	[illegible]
among groups	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
within groups	[illegible]	[illegible]	[illegible]			[illegible]
total	[illegible]	[illegible]				

[illegible]	SS	df	MS	Fs	p	[illegible]
among groups	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
within groups	[illegible]	[illegible]	[illegible]			[illegible]
total	[illegible]	[illegible]				

[illegible]	SS	df	MS	Fs	p	[illegible]
among groups	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
within groups	[illegible]	[illegible]	[illegible]			[illegible]

total	7193.955	332				

PR	SS	df	MS	Fs	p	vc (%)
among groups	315.482	3	105.161	15.262	0.0000	15.281
within groups	2266.942	329	6.890			84.719
total	2582.425	332				

LDR	SS	df	MS	Fs	p	vc (%)
among groups	18692.579	3	6230.860	142.941	0.0000	64.224
within groups	14341.222	329	43.590			35.776
total	33033.800	332				

LWR	SS	df	MS	Fs	p	vc (%)
among groups	153.511	3	51.170	4.130	0.0068	3.808
within groups	4076.587	329	12.391			96.192
total	4230.098	332				

NL	SS	df	MS	Fs	p	vc (%)
among groups	30.429	3	10.143	14.718	0.0000	14.785
within groups	226.732	329	0.689			85.215
total	257.161	332				

BSL	SS	df	MS	Fs	p	vc (%)
among groups	28.245	3	9.415	8.801	0.0000	8.980
within groups	351.969	329	1.070			91.020
total	380.215	332				

CL	SS	df	MS	Fs	p	vc (%)
among groups	6.140	3	2.047	0.525	0.6651	-0.604
within groups	1281.495	329	3.895			100.604
total	1287.634	332				

CW	SS	df	MS	Fs	p	vc (%)
among groups	32.915	3	10.972	2.188	0.0893	1.480
within groups	1649.885	329	5.015			98.520
total	1682.801	332				

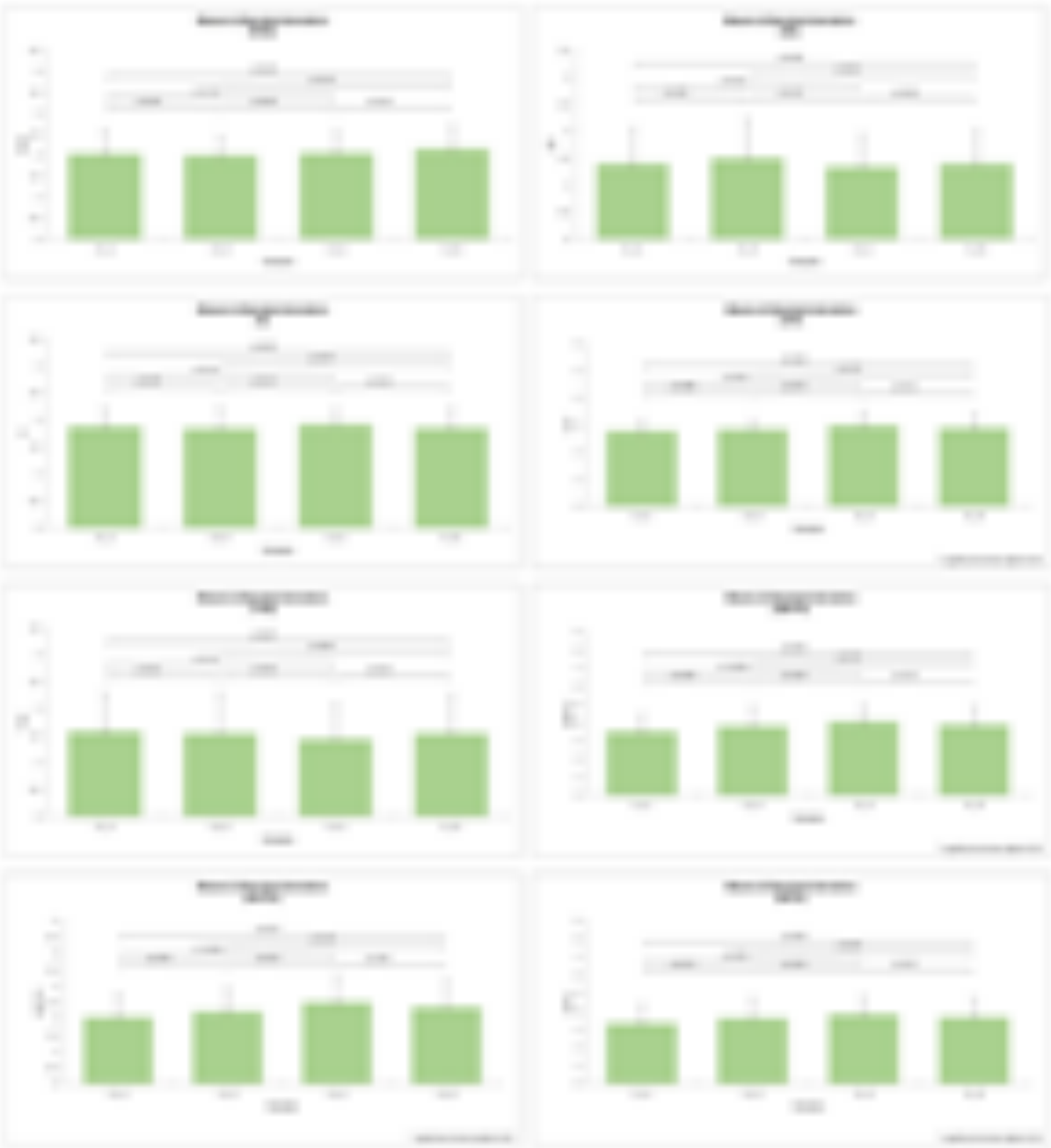
AL	SS	df	MS	Fs	p	vc (%)
among groups	382.118	3	127.373	5.757	0.0008	5.675
within groups	7278.807	329	22.124			94.325
total	7660.924	332				

AW	SS	df	MS	Fs	p	vc (%)
among groups	51.568	3	17.189	4.299	0.0054	4.005
within groups	1315.505	329	3.998			95.995
total	1367.073	332				

CW/CL	SS	df	MS	Fs	p	vc (%)
among groups	0.082	3	0.027	0.369	0.7752	-0.804
within groups	24.347	329	0.074			100.804
total	24.429	332				

CL/AL	SS	df	MS	Fs	p	vc (%)
among groups	0.415	3	0.138	4.346	0.0051	4.060
within groups	10.475	329	0.032			95.940
total	10.890	332				

Online Resource 5 Kruskal-Wallis results for the morphological leaf and fruit characters in the four groups identified by cluster analysis.



Introduction to the Molecular variability

In this chapter, extensive molecular analyses were carried out using different genetic markers. The purpose was to provide a genetic analysis to be compared to the taxonomical ones. To achieve this aim a set of markers already used by other researchers to study the *Fagaceae* family and in particular, the *Quercus* genus was used (Manos et al., 2001; Durand et al., 2010; Kremer et al., 2007, 2012; Hubert et al., 2014; Sullivan et al., 2016). In some of these studies, oak species were taken as a model to investigate the genomic delineation of critical oak taxa and possible events of microevolution which were occurring within this genus (Kremer et al., 2012; Kremer & Hipp, 2019).

Durand et al. (2010) presented a large catalogue of Expressed Sequence Tags (ESTs) developed from cDNA libraries *'to obtain expressional sequence information in contrasting environmental conditions or across developmental stages'*. The same authors argued that this catalogue was an inexpensive source of gene-based DNA markers, primarily to obtain SSRs. These molecules, ESTs, are fragments of coding DNA, that show flanking sequences of EST-SSRs located in well-conserved DNA regions in phylogenetically related species. This evidence makes them the *'marker of choice for comparative mapping and relevant functional and positional candidate genes to study their co-location with quantitative trait loci (QTLs)'*. The high degree of transferability of SSRs across species makes them very useful markers for aligning different genetic maps, as in *Q. robur*. Moreover, SSRs were used for comparative mapping analysis among other critical *Fagaceae* taxa. (ibid.) It is well-known that the hybridisation is a common event in the genomic evolution of *Fagaceae* (Kremer & Hipp, 2019). It is considered as frequently occurring among the various species belonging to the European white oaks (sections *Quercus*), as demonstrated by the large number of papers published over the last two decades (Ishida et al., 2003; Curtu et al., 2007a,b; Lepais & Gerber, 2011; Castillo-Mendoza et al., 2019; Kremer & Hipp, 2019).

The findings concerning natural populations of white oaks showing peculiar morphological features of their diagnostic characters (e.g. intermediate characters among two oak taxa or even more than two) have often been interpreted as an expression of hybridisation events. Hybridisation can be considered the crossbreeding between individuals of different species, and it is often cited as introgressive hybridisation or introgression. This latter phenomenon is represented by the transfer of genes between species led by backcrossing (see Anderson, 1949; Arnold, 1997; Rieseberg & Carney, 1998). In the other words, introgression is the occurrence of gene flow from one population belonging to a species into the gene pool of another population, through different events led by the backcrossing of an interspecific hybrid with one of its parent species.

This phenomenon creates an allelic frequency variation and can transfer adaptively genetic variations (Choler et al., 2004; Martin et al., 2006; Castric et al., 2008; Kim et al., 2008; Twyford & Ennos, 2012), demonstrating that hybridisation and introgression are two of the most relevant phenomena in the microevolutionary history of oaks (Lepais, 2009; Kremer & Hipp, 2019).

Such a microevolutionary event is based on changes in allele frequencies within a population over time. Four processes are mainly involved in this genetic issue: mutation, selection (natural and artificial), gene flow and genetic drift (Briggs, 2009). Microevolutionary events are linked with the sympatric of population, and, under such conditions, there is the possibility of the occurrence of a hybrid zone area, a surface where two closely related species are in reproductive contact, producing a range of hybrid and backcrossed entities. Generally, these conditions occur where great upheavals in ecosystems produce a wide range of different habitats (Briggs, 2009).

Great upheavals in oak forest ecosystems occurred many times in the past due to consequences of climate change in the glacial and post-glacial ages or due to more recent human activities. Where some new habitats or niches form, the hybrids can have a selective advantage in the ecosystem, as some of their new traits afford them a selection success. In this way, they increase their fitness, increase their presence in the population and may trigger a speciation episode (Briggs, 2009). When we observe a set of microevolutionary processes at a regional and global geographic-scale in a long time-scale, we speak of macroevolution events. Such macroevolutionary mechanisms involve the evolution process above the species level. In this way, the target of the macroevolutionary study is not a single species, but rather the assessment of the diversity of the entire clade and its position on the phylogenetic tree of this group.

The role of hybridisation in triggering some evolution processes is still a controversial topic. Some authors have suggested that hybridisation plays a crucial role in the formation of new species, in the transfer of adaptations and in generating a gene flow of one species into another species (Arnold et al., 2010), while some recent researchers have noted that hybridisation can also maintain a level of species integrity (Kremer & Hipp, 2019). Regarding this topic, oaks have become model taxa in the investigation of the potential role of hybridisation and introgression in adaptive evolution (Aldrich & Cavender-Bares, 2011; Lind & Gailing, 2013).

About the *'maintain a level of species integrity'*, Kremer & Hipp (2019) argue that this condition can involve *'even only a limited part of the genome, comprising numerous widely distributed small regions, is responsible for maintaining species barriers'* (Eaton et al., 2015; Leroy et al. 2017, 2019). According to some authors (Cavender-Bares, 2019; Leroy et al., 2019), hybridisation can be seen *'as a microevolutionary process reinforcing adaptation, and, more surprisingly, enhancing migration (Petit et al., 2003), as shown by ecological and population genomics studies of various oak complexes'* (Kremer & Hipp, 2019).

However, as a consequence of allelic rearrangement due to the low reproductive barriers among species, we observe the appearance of new phenotypes within populations (Arnold et al., 1995; Rieseberg & Gerber 1995; Barton 2001; Curtu et al., 2017). In the oak group, the lack of success of the reproductive isolation barriers, pre-zygotic (different timing of flowering, the competition during the germination of pollen grains or fertilization) or post-zygotic (e.g., reduced viability, death or sterility of hybrids, inefficient reproductive behaviour), seems rather recurrent, especially, as, in oaks, *'pre-zygotic barriers make the greatest contribution to reproductive isolation'* (Lepais & Gerber, 2011; Jurkšienė et al., 2019).

The genetic phenomena (hybridisation, introgression, gene flow) could be had interested also the group of the Italian pubescent oaks, giving rise to the high morphological variability that is at the basis of the high degree of uncertainty regarding their identification and classification.

What is emerging at present, is that there are no singular characters that can unambiguously be used to distinguish *Q. virgiliana, Q. dalechampii, Q. congesta, Q. ichnusae, Q. leptobalana, Q. amplifolia* from each-other (Bussotti & Grossoni, 1997; Borazan & Babac, 2003; Wellstein & Spada, 2015; Musarella et al., 2018; Di Pietro et al., 2016, 2019). Unlike, we are quite sure about the identification of other white species such as *Q. frainetto*, *Q. pubescens*, *Q. petraea*, and *Q. robur* (Fortini et al., 2009; Viscosi et al., 2009, 2011, 2012; Kätzel et al., 2014; Fortini et al., 2015; Antonecchia et al., 2015; Crãciunesc et al., 2016; Rellstab et al., 2016). Regarding *Q. petraea*, and *Q. robur*, these two species are morphologically very similar mainly as regards the leaves. However, many studies have demonstrated that it is possibly recognised the occurrence of intermediate forms (maybe hybrid) and pure forms of individuals (belonging at the putative species) by using various multivariate analyses procedures (Aas, 1993; Bacilieri et al., 1994; Kremer et al., 2002; Boratynski et al., 2008; Crãciunesc et al., 2016).

For all these reasons, it is clear that a classical biological concept of species based on reproductive barriers does not fit well for the group of oaks (Van Valen, 1976), given the normal distribution of morphological variables among individuals and population. This critical group of oaks requires a multidisciplinary approach to assess the relationship between different taxa.

Some authors suggested that multivariate analyses, based on a set of morphometric traits, can often be used to determinate among closely related interfertile species (Jensen et al., 1993; Kremer et al., 2002; Curtu et al., 2007a; Gailing et al., 2012; Viscosi et al., 2012; Yucedag & Gailing 2013; Gailing & Curtu 2014). The classifications based on the molecular studies (Manos et al., 2008; Denk & Grimm, 2010; Hubert et al., 2014; Denk et al., 2017; Hipp et al. 2015, 2019) have clarified the infrageneric division of genus *Quercus*, demonstrating also that the *'differentiation within clades'* of the morphological traits has followed the ecological and climatic niche evolution (Hipp et al. 2019). However, also if on the last ten years have been published many studies around the white oaks many taxonomic doubts remain about the classifications at the rank of species.

Submitted

Chapter 4. Does the genetic diversity among pubescent white oaks in southern Italy, Sicily and Sardinia Islands support the current taxonomic classification?[13]

Romeo Di Pietro[1], Antonio Luca Conte[2], Piera Di Marzio[2], Paola Fortini[2*], Emanuele Farris[3], Lorenzo Gianguzzi[4], Markus Müller[5], Leonardo Rosati[6], Giovanni Spampinato[7], Oliver Gailing[5].

[1]Department PDTA, University of Rome Sapienza, 00196 Rome, Italy.
[2]Department of Bioscience and Territory, University of Molise, 86090 Pesche (IS) Italy.
[3]Department of Science and Territory, University of Sassari, I-07100 Sassari, Italy
[4]Department of Agricultural and Forest Sciences, Università degli Studi di Palermo, I-90123 Palermo, Italy.
[5]Faculty of Forest Sciences and Forest Ecology, Forest Genetics and Forest Tree Breeding, University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany.
[6]School of Agricultural, Forestry and Environmental Sciences, University of Basilicata, I-8500 Potenza, Italy.
[7]Department of Agriculture, Mediterranean University of Reggio Calabria I- 89122 Reggio Calabria, Italy.

✉ Corresponding author, Paola Fortini: fortini@unimol.it

Abstract

Molecular diversity analysis of deciduous pubescent oaks from the southernmost end of the Italian peninsula, Calabria, Sicily and Sardinia were carried out. The aims of this study are twofold. First, to provide data on the genetic diversity of white pubescent oaks from an area that is unknown from this point of view and that currently exhibits one of the highest concentrations of pubescent oak species in the whole Europe. Second, to verify if this groups of oak were distinguishable on the basis of their genetic diversity and if their identification is in accordance with the current taxonomic classification. Molecular analyses of leaf material of 489 trees from seventeen populations belonging to different pubescent oak species (*Quercus amplifolia, Q. congesta, Q. dalechampii, Q. ichnusae, Q. leptobalanos, Q. virgiliana*) were performed.

Twelve gene-based expressed sequence tag-simple sequence repeat markers, were used to arrange a polymerase chain reaction. Genetic diversity parameters were calculated for each locus within population. The results showed relatively high values of allelic richness, heterozygosity and number of private alleles for the populations investigated. A weak but positive correspondence between

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[13] This part of thesis has been submitted as an article on the European Journal of Forest Research (Springer). And in this thesis has reported in the same model submitted at the journal. The tables and figures are related to this part of the text and not to the general numbering followed for the other chapters of the thesis.

geographical and genetic distance was highlighted. Multivariate statistic and genetic assignment analyses revealed no significant separation among the oak populations investigated, suggesting their probable belonging to a single taxon. In light of the results obtained, the taxonomic classification for the pubescent white oaks currently reported in the major Italian floras and checklists for the study area would not seem to be confirmed by molecular analyses.

Keywords: Bayesian analysis, Genetic variation, Nuclear microsatellites EST-SSRs, pubescent oaks, Taxonomy.

Introduction

The deciduous oak woods represent the most abundant forest vegetation type in southern Europe (Mucina et al. 2016). In the Italian Peninsula these are dominant throughout the whole Apennine range with an increase in the sclerophyllic evergreens oak component (*Quercus ilex, Q. suber* and *Q. coccifera*/*Q. calliprinos*) moving southwards (Blasi and Di Pietro 1998; Blasi et al. 2004; Di Pietro et al. 2010). However, even in the southernmost end of Italy and Sicily the thermophilous deciduous oak forests cover a wider area than that covered by evergreens oak forests (Gianguzzi et al. 2015) whereas the opposite is true for Sardinia Island where only 15% of the territory is potentially covered by deciduous oaks (Bacchetta et al. 2009). Both taxonomic and phytosociological literature report that the thermophilous broad-leaved forests of southern Italy, Sicily and Sardinia are characterised by different pubescent oak species living in sympatric conditions. The pubescent oaks in issue belong to the white oaks (Subgenus *Quercus*; Section *Quercus*) and are characterised by pubescent leaves and twigs that allow them to be distinguished by other white oak species such as *Q. petraea* and *Q. robur*. The high concentration of pubescent white oak species in the study area could find valid reasons in the fact that southern Italy, Sicily and Sardinia acted as primary refugia for the oak forest vegetation during the Quaternary cold periods (Sadori and Narcisi 2001; Fineschi et al. 2004). It follows the well-established theory according to which several thermophilous tree species escaped from central and northern Europe to survive in the coastal and hilly belts of the Iberian, Italian and Balkan Peninsulas (Huntley and Birks 1983; Watts et al. 1996; Brewer et al. 2002; Tzedakis et al. 2002). Furthermore, the degree of geographic isolation may have played a non-marginal role in the current degree of phenotypic diversification of the pubescent oaks of the study area. Southern Calabria is a narrow mountainous promontory dividing Tyrrhenian and Ionian Seas, while Sicily and Sardinia are the largest Mediterranean Islands that experienced different paleogeographic vicissitudes - as testified by their different type of floristic endemic component (Arrigoni et al. 1977-1991; Medail and Quezel 1997; Brullo et al. 2011; Pignatti 2011; Sciandrello et al. 2015) - which may have affected the evolution of the *Quercus* genetic pools in a different way (see Petit et al. 2002b; Fineschi et al. 2004).

On the basis of the most recently published National floras (e.g. Brullo et al. 1999; Pignatti et al. 2017-2019) seven pubescent oaks are considered as occurring in southern Italy. These are: *Q. amplifolia* Guss., *Q. congesta* C. Presl., *Q. dalechampii* Ten., *Q. ichnusae* Mossa, Bacch. and Brullo, *Q. leptobalana* Guss., *Quercus pubescens* Willd. and *Q. virgiliana* Ten. In the recent checklist of the Italian vascular Flora (Bartolucci et al. 2018) only four of these species are considered as valid names (*Q. pubescens*, *Q. dalechampii*, *Q. congesta* and *Q. ichnusae*) the remaining three being considered as synonyms. It is noteworthy that Sicily, Sardinia and southern Calabria are *locus classicus* for five of the aforementioned seven pubescent-oak species and that some of these species (e.g., *Q. congesta*, *Q. dalechampii* and *Q. virgiliana*) are considered "good species" not only in Italy but also in several other European countries. Nonetheless, the debate on the real taxonomic value of all these pubescent oak species is still very heated throughout Europe although there are few published studies that addressed the problem with a multidisciplinary approach also considering molecular data (Fraijnic et al. 2005; Di Pietro et al. 2016, 2020; Musarella et al. 2018).

The aim of this paper is twofold. First, to provide first insights in the genetic diversity from an area that, although being considered as highly important for the European white oaks diversity, has not been characterised at genetic markers. Second, to verify if groups of oak individuals (or populations) are distinguishable on the basis of their genetic features in order to confirm, or support the assumption of the occurrence of different pubescent oak species in a territory with a high biogeographical value such as the one comprising Sicily and Sardinia Islands and the southern Calabria.

Material and Methods

Study area

This study was carried out in Southern Italy, in mixed deciduous forest habitats which are located in administrative regions of Calabria (the southernmost end of this region included between the Serre Calabre and the Aspromonte massifs), Sicily and Sardinia (41° 18′ N - 7° 23′ E; 36° 19′ N - 18° 16′ E) (Fig. 1).

Fig. 1 Study sites in Calabria, Sardinia and Sicily.

Oak tree material

Leaf material of 17 pubescent-oak populations was collected during autumn of 2017 and 2018. The collections were made in oak populations whose tree guide species were already identified and abundance indexes already published in the phytosociological tables of the following associations:, *Erico-Quercetum virgilianae, Lonicero-Quercetum virgilianae, Oleo-Quercetum virgilianae Ornithogalo-Quercetum ichnusae, Glechomo-Quercetum congestae, Arabido-Quercetum congestae, Quercetum leptobalanos* (Brullo 1983; Brullo and Marcenò 1984; Brullo et al. 2001; Brullo et al. 2008; Bacchetta et al. 2009) (Table 1).

Table 1 Geographic features of the 17 oak populations sampled in Calabria, Sardinia and Sicily

Stand ID	No. of samples	Coordinates GMS D° M' S”	Location	Region	Guide species	Altitude (m a.s.l.)
SIC01	30	37°51'55 98” N 13°23’13.68” E	Bosco Ficuzza (Corleone, Palermo)	Sicily	*Quercus leptobalana* Guss.	919
SIC02	30	37°56'50 59” N 13°23’43.71" E	Santuario (Marineo, Palermo)	Sicily	*Quercus virgiliana* (Ten.) Ten.	459
SIC03	30	37°48’40.30" N 15° 4’56.26" E	Etna, SP Mare-Neve presso Chalet delle Ginestre	Sicily	*Quercus congesta* C.Presl.	1298
SIC04	30	37°44’19.60" N 15° 6’18.64" E	Etna, SP Mare-Neve sopra Fornazzo	Sicily	*Quercus dalechampii* Ten.	900
SIC05	29	37°35’32.67" N 15° 2’42.61" E	Etna, Monte Ceraulo, Mascalucia (Catania)	Sicily	*Quercus virgiliana* (Ten.) Ten.	538
SIC06	31	37°36’29.87" N 15° 4’17.95" E	Etna, Trecastagni via P. Togliatti (Catania)	Sicily	*Quercus virgiliana* (Ten.) Ten.	544
CAL07	28	38°36’57.93" N 16°10’17.16" E	Serre, Sant’Angelo SS 182 bivio SP per Pizzoni (VV)	Calabria	*Quercus dalechampii* Ten.	260
CAL08	32	38°29’16.81" N 16°22’11.31" E	Serre, SS 110 bivio SP 90 per Nardidipace (VV)	Calabria	*Quercus congesta* C.Presl.	1190
CAL09	29	38°22’24.18" N 15°55’49.69" E	Aspromonte, SP Palmi Pontevecchio - Croce Mammone, presso Cirello, Rizziconi (RC)	Calabria	*Quercus dalechampii* Ten.	70
CAL10	31	38°13’9.00" N 15°53’16.61" E	Aspromonte SP 3 (ex SS 183) bivio Piani di Carmelia (RC)	Calabria	*Quercus congesta* C.Presl.	980
SIC11	34	37°54'36 56” N 13°59’06.12" E	Madonie tra Piano Torre e Piano Zucchi (Collesano, Palermo	Sicily	*Quercus leptobalana* Guss.	864
SIC12	30	38°03'51 68” N 14°45’01.68" E	Nebrodi, Valle del Fiume Fitalia (Frazzanò, Messina)	Sicily	*Quercus virgiliana* (Ten.) Ten.	246
SIC13	32	37°56'29 75” N / 14°52’34.38” E 37°57'07 84” N / 14°52’15.22” E	Nebrodi, Foresta (Bosco del Flascio)	Sicily	*Quercus congesta* C.Presl.	1162-1172
SAR14	30	39°22’38.15"N 9° 3'33.50"E	M.te Zara, Monastir (CA)	Sardinia	*Quercus pubescens* Willd.	130-167
SAR15	30	40°14’8.22"N 8°40'47.49"E	Monte Sant’Antonio, Macomer (NU)	Sardinia	*Quercus ichnusae* Mossa, Bacch. & Brullo	780
SAR16	25	40°45’5.38"N 8°31'27.82"E	Sant’Orsola, Sassari (SS)	Sardinia	*Quercus virgiliana* (Ten.) Ten.	138
SAR17	15	40°25’39.90"N 9° 0'24.74"E	Monte Rasu, catena del Goceano, Bono (SS)	Sardinia	*Quercus congesta* C.Presl.	1098 - 1197

Sites of collections SIC3, CAL9, SIC11, SAR15 were located in the proximity of the '*loci classici'* of *Q. congesta, Q. dalechampii, Q. leptobalana* and *Q. ichnusae*, respectively.

A total of 489 oak trees were analysed. At each stand, leaves were collected from a minimum of 15 and a maximum of 34 tree individuals. The minimum distance between collected trees was at least 30 m.

DNA extraction

For all the samples coming from Calabria and Sicily (a total of 393 samples), the DNA was extracted from leaves using the Invisorb® Spin Plant Mini Kit (INVITEK Molecular GmbH, Berlin, Germany) and the work was carried out in the Plant Biology Laboratory of DIBT of Isernia (Italy). For those samples coming from Sardinia (96 samples), DNA was extracted using the Qiagen DNeasy 96 plant kit (Qiagen, Hilden, Germany) and the work was carried out in the Forest Genetics and Forest Tree Breeding Laboratory at the University of Göttingen (Germany).

Twelve gene-based microsatellite markers (EST-SSRs) were used: PIE239, PIE227, PIE223, PIE215, PIE020, PIE152, PIE243, PIE242, PIE267, PIE102, PIE258, PIE271 (Durand et al. 2010).

This set of EST-SSRs markers (PIE) was chosen according to other recent 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. 2020). The primer pairs were combined into three different multiplex reactions, called Mu1, Mu2 and Mu3 (Table 2). The dye type and primers are also shown in Table 2. A PCR Mastermix was obtained blending 1 µL DNA, 1.5 µL 10× reaction buffer B (Solis BioDyne, Tartu, Estonia), 1.5 µL $MgCl_2$ (25 mM), 1 µL dNTPs (2.5 mM each dNTP), and 0.2 µL (5 U/µL) HOT FIREPol *Taq* DNA polymerase (Solis BioDyne, Tartu, Estonia). To this admixture, we have added EST-SSR primers. The volume, concentration and dye labels of each primer are shown in Table 2.

Table 2 Primers combinations and information about the different multiplex (Mu) reactions

	SSR ID	Observed size range (bp)	Motif type	Forward primer (5'-3')	Reverse primer (5'-3')	Primer Concentr. [5 picomole/µL]	Repeat no.	Expected size (bp)	Dye type
Mu1	PIE239	80 - 115	$(AT)_{12}$	CAACAAATGGCTCAACAGTG	CCCATTTGGTAGCAAAGAGTC	1 µL	12	95	6-FAM
	PIE227	140 - 175	$(TGG)_8$	ACCATGATCTGGGAAGCAAC	AAGGGCTTGGTTGGGTTAGT	0.5 µL	8	160	6-FAM
	PIE223	180 - 240	$(GGT)_8$	AGAAGCCCAACACGGCTAC	AGCAAAACACAAACGCACAA	1 µL	8	200	FAM
	PIE215	180 - 235	$(GAG)_6$	ACGAAATGGAGCTGTTGACC	TCTCCTTCTCTTCTGCCATGA	1 µL	6	200	HEX
Mu2	PIE020	94 - 123	$(TA)_{12}$	GCAGAGGCTCTTCTAAATACAGAACT	GGGAGGTTTCTGGGAGAGAT	0.5 µL	12	180	6-FAM
	PIE152	228 - 265	$(AG)_{11}$	TGTACCTCTTTCCTCTCTCTAAAACT	GAATTTCTAAACCACTAGCATTGAC	0.5 µL	11	247	HEX
	PIE243	200 - 236	$(AG)_{15}$	GGGGTCAGTAGGCAAGTCTTC	GAGCTGCATATTTTCCTTAGTCAG	0.5 µL	15	220	6-FAM
	PIE242	95 - 129	$(TA)_{10}$	GGAGGGAAAAGAACAATGC	TTGCAATCCTCCAAATTTAATG	0.5 µL	10	113	HEX
	PIE267	85 - 105	$(AG)_{11}$	CCAACCATCAAGGCCATTAC	GTGCGAACAGATCCCTTGTC	0.5 µL	11	100	6-FAM
	PIE102	130 - 180	$(AG)_{12}$	ACCTTCCATGCTCAAAGATG	GCTGGTGATACAAGTGTTTGG	0.5 µL	12	160	HEX

Mu3	PIE258	120 - 180	$(TC)_{13}$	TCTCGATCTCAAAACAAAACCA	TTTGATTTGTTTAAGGAAAATTGGA	0.5 µL	13	150	6-FAM
	PIE271	181 - 230	$(TC)_{11}$	CACACTCACCAACCCTACCC	GTGCGGTTGTAGACGGAGAT	0.5 µL	11	190	HEX

The PCR reactions were conducted using a touchdown program as follows: denaturation at 95 °C for 15 min, followed by 10 touchdown cycles of 94 °C for 1 min, 60 °C (−1 °C per cycle) for 1 min, and 72 °C for 1 min. The second step consisted of 25 cycles at 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min, followed by a final extension step of 72 °C for 20 min. PCR reactions were performed in a DNA Biometra Thermocycler TOptical Gradient 96 (Biometra, Goettingen, D, EU). The subsequent separation of fragments was performed using GS 500 ROX (Applied Biosystems, Foster City, USA) as size standard in an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, USA). Allele scoring was done with the GeneMapper 4.0 software (Applied Biosystems, Foster City, CA, USA).

Genetic assignment

The main genetic statistics were obtained using GenAlEx software v. 6.5 (Peakall and Smouse 2012). With this software we calculated all the basic molecular statistics, the mean number of alleles (N_a), observed heterozygosity (H_o), expected heterozygosity (H_e) and fixation index (G_{ST}) per locus and population, and inbreeding coefficient (F_{IS}) per locus. We have also calculated pairwise G_{ST} values between populations based on 1000 permutations, and this data set (Pairwise Population Matrix of G_{ST} Values) was also used to perform a Principal Coordinates Analysis (PCoA) based on covariance with data standardisation (using the tri distance matrix). Using GenAlEx software v. 6.5 we also tested for significant correlations between pair-wise co-dominant genotypic distance and geographical distance by applying simple Mantel tests with 9,999 permutations.

FSTAT v. 2.9.4 (June 2003) was used for obtaining allelic richness (A_r), and the inbreeding coefficient (F_{IS}) per population using 1000 permutations to test for significant differences from “zero”. Number of alleles per locus (K), null allele frequencies (F_{null}), polymorphic information content (PIC) and deviations from Hardy-Weinberg Equilibrium (HW) were calculated using Cervus 3.0.7 (Marshall et al. 1998).

Analysis of molecular variance (AMOVA) was performed with Arlequin v. 3.5.2.2 (Excoffier et al. 2010). In addition, we have obtained a matrix of distances based on the chord genetic distance of Cavalli-Sforza and Edwards (1967), with 1000 bootstraps on loci, using Populations v. 1.2.32 software (Langella 1999). MEGA 7.0.26 software (Kumar et al. 2016) was used to display the tree.

In order to infer molecular clusters and to assign individuals to populations, STRUCTURE v. 2.3.4 software based on the Bayesian clustering method was used (Pritchard et al. 2000). We performed genetic analysis with STRUCTURE under the admixture model without prior information about the location or taxonomical classification and applying the correlated allele frequency model. The degree admixture “Alpha” has been set to be inferred from the data while “Lambda”, the parameter of the distribution of allelic frequencies, has been set to one. To assess the number of clusters that best fit the data, a burn-in period of 50,000 and Markov chain Monte Carlo (MCMC) simulations of 100,000 were used, considering values of K from one to ten, with ten replications for each value of K. We have visualised the log-likelihoods over different values of K (Evanno et al. 2005) with STRUCTURE

HARVESTER (Earl, 2012) while the graphical representation and a summary of the STRUCTURE results were obtained using the CLUMPAK software (Kopelman et al. 2015).

Results

Molecular statistics analysis

The analysis exhibited a mean of 7.9 different alleles per locus (N_a) for a total of 169 alleles across all populations (Table 3). The locus that exhibited the highest number of alleles is PIE102 with 20 alleles. The mean number of alleles (N_a) for all populations ranged from 2.9 (PIE227) to 11.5 (PIE152), the observed heterozygosity (H_o) ranged from 0.227 (PIE227) to 0.834 (PIE271), and the expected heterozygosity (H_e) ranged from 0.208 (PIE227) to 0.864 (PIE152). PIE227 exhibited the lowest value for N_a, H_o, H_e among all loci. The F_{IS} values were significantly different from zero for four loci and ranged from -0.005 (PIE020) to 0.398 (PIE239). High and positive F_{IS} values and significant evidence for both null alleles were only detected for PIE239 and PIE258.

Table 3 Sample size and mean genetic diversity indices over all the 17 populations sampled in Calabria, Sardinia and Sicily

Marker	N	K	N_a	H_o	H_e	F_{IS}	G_{ST}	PIC	F(Null)	HW
pie020	474	13	5.9	0.516	0.513	-0.005	0.023*	0.494	0.0137	NS
pie102	470	20	10.1	0.766	0.750	-0.021	0.025*	0.760	0.0139	NS
pie152	469	18	11.5	0.819	0.864	0.052	0.023*	0.893	0.0486	NS
pie215	471	14	9.4	0.819	0.796	-0.029	0.010*	0.800	0.0018	NS
pie223	471	12	8.8	0.813	0.818	0.007	0.030*	0.842	0.0253	NS
pie227	468	8	2.9	0.227	0.208	-0.089	0.068*	0.230	0.0031	NS
pie239	456	14	5.1	0.249	0.414	0.398	0.063*	0.453	0.3013	***
pie242	471	16	9.2	0.790	0.813	0.028	0.039*	0.845	0.0416	*
pie243	473	15	6.4	0.612	0.645	0.051	0.034*	0.642	0.0532	NS
pie258	472	16	10.5	0.603	0.839	0.281	0.026*	0.873	0.1956	***
pie267	472	9	6.5	0.744	0.727	-0.023	0.030*	0.728	0.0138	NS
pie271	472	14	8.2	0.834	0.807	-0.034	0.029*	0.825	0.0053	NS
Mean	469.9	14.08	7.9	0.649	0.683	0.051	0.030*	0.699	-	-

N number of individuals, K number of alleles at the locus, N_a allelic arithmetic mean value across loci, H_o observed heterozygosity, H_e expected heterozygosity, F_{IS} inbreeding coefficient, G_{ST} fixation index (* $p<0.05$), *PIC* polymorphic information content, *F* (null) null allele frequency, *HW* Hardy-Weinberg equilibrium test (significance with Bonferroni correction: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Mean diversity indices across populations for each locus are shown in Table 4. The mean number of alleles (N_a) ranged from 6.5 in SAR17 (*Q. congesta* from West Sardinia, only 15 individuals) to 8.8 in both SIC01 and SIC13 (*Q. leptobalana* and *Q. congesta* from West and East Sicily, respectively) (total mean value 7.9). Mean allelic richness (A_r) ranged from 5.8 for SAR16 (*Q. virgiliana* - NW Sardinia) to 7.2 for SIC01 and SIC02 (*Q. leptobalana* and *Q. virgiliana* from West Sicily) whereas the total mean value was 6.7.

Table 4 Sample size and mean genetic diversity indices for the 17 populations sampled in Calabria, Sardinia and Sicily.

Population	Region	N	N_p	N_a	A_r	H_o	H_e	F_{IS}	G_{ST}	$P(G_{ST})$
SIC01	Sicily	30	5	8.8	7.2	0.632	0.709	0.126		
SIC02	Sicily	29	11	8.6	7.2	0.660	0.721	0.103		
SIC03	Sicily	27	1	7.7	6.7	0.625	0.687	0.110		
SIC04	Sicily	30	3	8.7	6.9	0.606	0.672	0.115		
SIC05	Sicily	26	1	8.2	6.7	0.646	0.683	0.073		
SIC06	Sicily	30	1	7.8	6.7	0.636	0.703	0.112		
SIC11	Sicily	33	2	8.3	6.9	0.676	0.711	0.065		
SIC12	Sicily	28	2	7.3	6.2	0.589	0.669	0.137		
SIC13	Sicily	32	23	8.8	7.0	0.682	0.727	0.077		
Mean		29	5.4	8.2	6.8	0.639	0.698	0.102		
Total		265	49				0.790 (H_T)		0.084	0.001
CAL07	Calabria	28	0	7.8	6.7	0.648	0.684	0.071		
CAL08	Calabria	32	0	7.8	6.5	0.677	0.694	0.040		
CAL09	Calabria	29	1	7.9	6.6	0.688	0.706	0.043		
CAL10	Calabria	30	5	8.1	6.9	0.623	0.696	0.121		
Mean		30	1.5	7.9	6.7	0.659	0.695	0.069		
Total		119	5				0.722 (H_T)		0.011	0.001
SAR14	Sardinia	30	2	6.8	6.1	0.633	0.630	0.011		
SAR15	Sardinia	30	4	8.3	6.8	0.672	0.652	-0.013		
SAR16	Sardinia	21	0	6.7	5.8	0.681	0.619	-0.075		
SAR17	Sardinia	15	3	6.5	6.4	0.660	0.645	0.011		
Mean		24	2.3	7.1	6.3	0.662	0.637	-0.017		
Total		96	9				0.687 (H_T)		0.037	0.001
General Mean		28	3.7	7.9	6.7	0.649	0.683	0.066		
Grand total		480	63	-	-	-	-	-		

N number of individuals, N_p number of private alleles, N_a number of alleles, A_r allelic richness, H_o observed heterozygosity, H_e expected heterozygosity, F_{IS} inbreeding coefficient, G_{ST} (analog of F_{ST} adjusted for bias) genetic differentiation among populations, $P(G_{ST})$ statistical significance of G_{ST}, H_T total expected heterozygosity

The observed heterozygosity (H_o) ranged from 0.589 for SIC12 (*Q. virgiliana* - NE Sicily) to 0.688 for CAL09 (*Q. dalechampii* - SW Calabria) with a total mean value of 0.649. The expected heterozygosity (H_e) ranged from 0.619 for SAR16 (*Q. virgiliana* - NW Sardinia) to 0.727 for SIC13 (*Q. congesta* - NE Sicily) with a total mean value of 0.683. The mean F_{IS} value was 0.066 while the minimum F_{IS} was -0.013 for SAR15 (*Q. ichnusae* - W Sardinia) and the maximum value was 0.137 for

SIC12 (NE Sicily). The total number of private alleles N_p found was 63. The highest number of private alleles (23) was found in SIC13 (*Q. congesta* – Sicily) while the second highest number was found in SIC02 (*Q. virgiliana* - Sicily) with 11 alleles. No private alleles were found in CAL07 and CAL08 (*Q. dalechampii* and *Q. congesta* from Calabria) and SAR16 (*Q. virgiliana* - NW Sardinia). The values of genetic diversity calculated for each administrative region (Calabria, Sicily and Sardinia) showed that the N_a values increased from Sardinia (7.1) to Calabria (7.9) and Sicily (8.2). A similar pattern was found for allelic richness (A_r) which was 6.3 for Sardinia, 6.7 for Calabria and 6.8 for Sicily. Mean H_e is lowest for Sardinian populations (0.637), while it is very similar for Sicilian (0.698) and Calabrian (0.695) populations. The ANOVA (Table 5) showed that differences in H_e values calculated among administrative regions were statistically significant whereas those in H_o were not.

Table 5 ANOVA for H_o and H_e in Calabria, Sardinia and Sicily. Tukey Kramer post-hoc test was performed, a and b letters meaning different group (significant for H_e).

Region	H_o	H_e
Calabria	0.659 a	0.695 b
Sardinia	0.662 a	0.637 a
Sicily	0.639 a	0.698 b
Pr > F(Model)	0.340	0.000
Significant	No	Yes

Values for H_e were significantly lower in Sardinia populations as compared to the Calabrian and Sicilian populations. It is possible that H_e data could be influenced by the lower total number of individuals and a lower number of individuals per population in two populations (21 individuals in SAR16 and 15 individuals in SAR17). However also the H_T values per region showed the lowest value for Sardinia and the highest for Sicily. The F_{IS} value was slightly negative for Sardinia (-0.017), while it was positive in Calabria and Sicily where it was found to be 0.069 and 0.102, respectively (Table 4).

The genetic distance (G_{ST}) within populations belonging to the same geographical region (Table 4) showed significantly higher values for Sicily (0.084) than those of Calabria and Sardinia (0.011 and 0.037 respectively). G_{ST} among geographical regions (Table 6) showed a higher degree of differentiation comparing the oak populations of Sardinia and Calabria (0.048) and Sardinia and Sicily (0.030) whereas lower values were found comparing the populations of Sicily and Calabria (0.013).

The highest pairwise genetic difference G_{ST} (Supplementary Table S1) was observed between SAR16 (*Q. virgiliana* - NW Sardinia) and CAL09 (*Q. dalechampii* - SW Calabria). Only one non-significant G_{ST} value was found in the pairwise matrix, between SIC05 (*Q. virgiliana* – Sicily) and SIC06 (*Q. virgiliana* – Sicily) (p-value 0.292).

Table 6 Pairwise matrix of G_{ST} values for Calabria, Sardinia and Sicily

	Sicily	Calabria	Sardinia
Sicily	0.000	0.001	0.001
Calabria	0.013	0.000	0.001
Sardinia	0.030	0.048	0.000

G_{ST} values below the diagonal. Probability, based on 999 permutations, is shown above the diagonal.

These are two oak populations located at similar altitudes at the base of Etna volcano and distant about 4 km from each other as the crow flies. The PCoA (Fig. 2) showed that all the populations from Sardinia (especially SAR16) segregated in the right part of the diagram, far away from the other populations investigated. Populations coming from Sicily and Calabria formed a mixed group in the left side of the diagram according to a distribution pattern, which does not seem to follow any identifiable criterion.

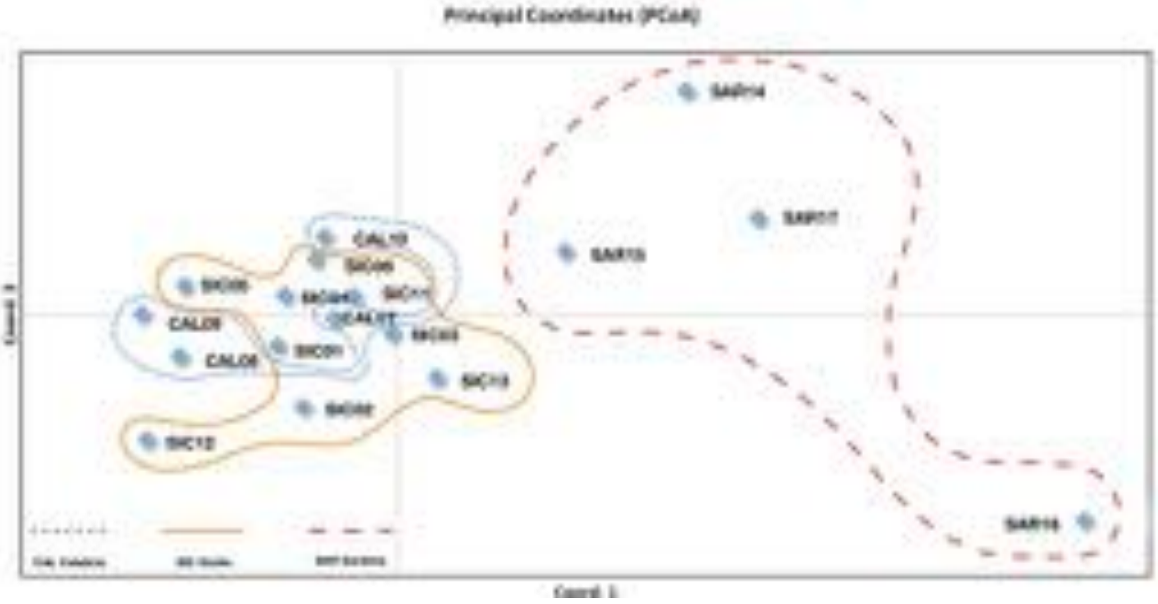

Fig. 2 Principal coordinate analysis (PCoA) of the 17 populations sampled in Calabria, Sardinia and Sicily (coordinate 1 and coordinate 2 explain 33.31% and 15.94% of the variation between populations, respectively).

The Mantel test (Fig. 3) showed a positive, although weak, correlation ($R^2 = 0.085$; $p=0.001$) between genetic distance (Gen by POP GD) and geographic distance (Geographic POP GGD) of populations.

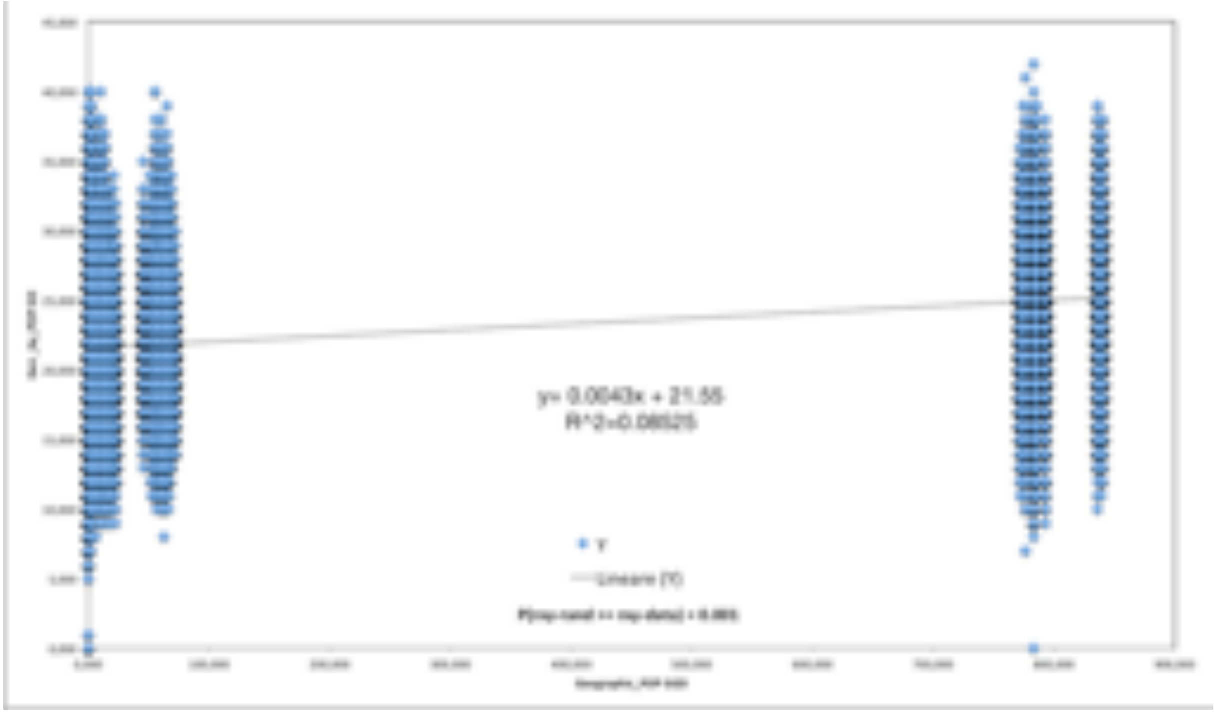

Fig. 3 Isolation-by-distance patterns for individuals, plotting pairwise codominant genotypic distance (Gen by POP GD) versus pairwise geographic distances (Geographic POP GGD). The figure includes statistical significance (p=0.001) obtained by simple Mantel tests in GenAlEx, version 6.5. Each point (diamond) represents a pair-wise comparison.

According to the global AMOVA (Table 7), most of the genetic variation (92.05% percentage of variation) was found “within individuals” (p-value 0.001), followed by that “among individuals within populations” (5.15%) and “among populations” (2.80%).

Table 7 AMOVA results as weighted average over loci for the 17 populations sampled in Calabria, Sardinia and Sicily.

Source of variation	Degree of freedom	Sum of squares	Variance components	Percentage of variation	Probability
Among populations	16	173.084	0.011644	2.80	0.0001
Among individuals within populations	463	1968.462	0.29870	5.15	0.0001
Within individuals	480	1835.500	3.82396	92.05	0.0001
Total	959	3977.046	4.1541		
Fixation indices	F_{IS} 0.05295	F_{ST} 0.02803	F_{IT} 0.07949		

The Neighbor joining-based tree showed the occurrence of three main clusters (A, B and C) exhibiting a low degree of significance (Fig. 4). Group A is divided into two main subgroups, one of which (A1 bootstrap 8) is composed of all the Calabrian populations (CAL07-10) and the other (A2 bootstrap 79) of two Sicilian populations (SIC05 and SIC06) located very close to each other geographically. Group B comprises two Sicilian populations, one of these (SIC03) referred to a *Q. congesta* population from the montane belt of Etna volcano and the other (SIC12) to a *Q. congesta* population of the lower hilly belt of Nebrodi Mountains. Group C is the most numerous and is composed of a well differentiated subgroup (C1), which includes the four Sardinian populations (bootstrap 51) and a set of single Sicilian populations that segregate more or less individually, except for SIC01 and SIC02 with a bootstrap value of 50. Genetic distances, which characterize the three main groups and the four further subgroups, are very low except for the Sardinian subgroup (C1).

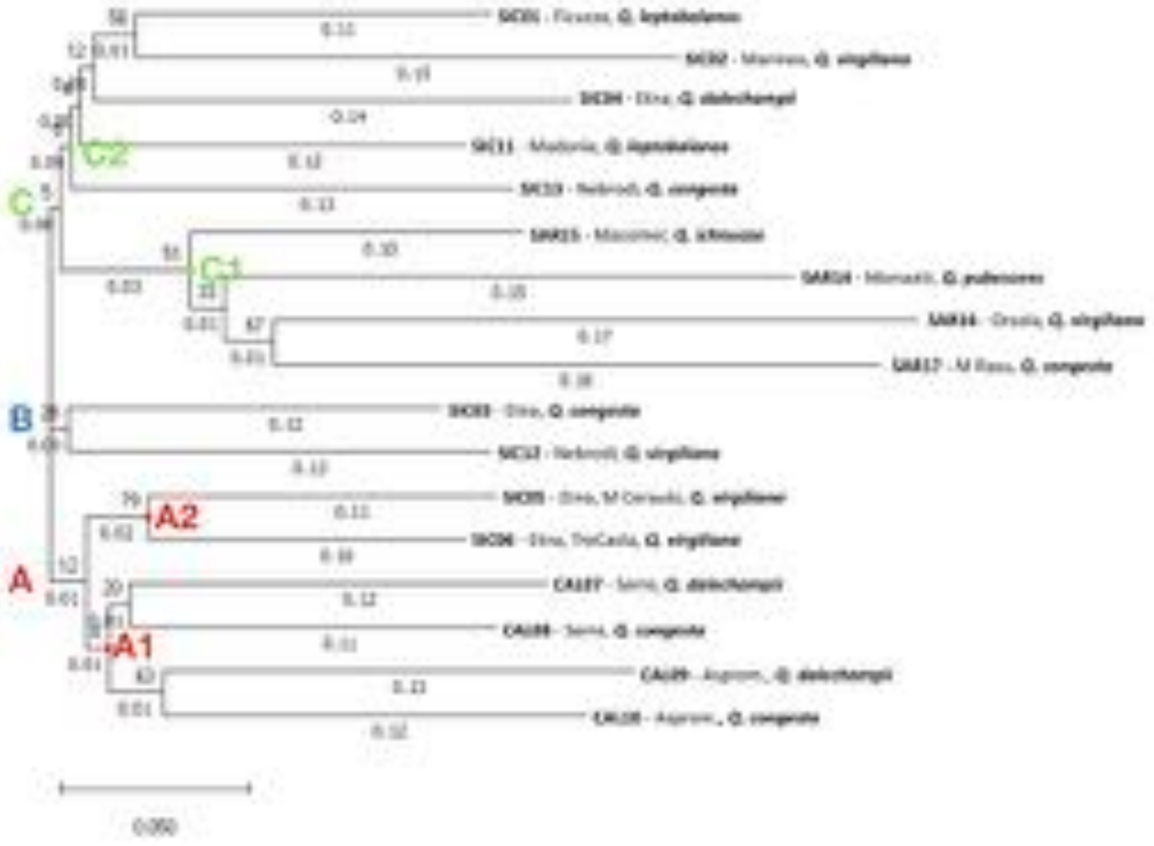

Fig. 4 Neighbor-joining (NJT) of the 17 populations sampled in Calabria, Sardinia and Sicily based on the chord genetic distance of Cavalli-Sforza and Edwards (1967)

The generally low bootstrap values indicate no phylogenetic signal suggesting that all populations represent a single taxon.

The Bayesian analysis revealed K=8 as the most probable number of clusters obtained with the ad hoc statistic ΔK. According to ad hoc statistic ΔK (Supplementary Figure S1**),** we obtained lower significant peaks also for K=2 and K=4. Only three out of 480 samples analysed exhibited Q values >0.90 all coming from Sardinia (2 samples from SAR14 and 1 sample from SAR16). Twenty-tree samples exhibited a Q value >0.80, ranging between 0.807 and 0.898. These individuals were found to be distributed as follows: seventeen from Sardinia (7 samples from SAR16 and 10 from SAR14) four from Sicily (2 from SIC5, 1 from SIC12, 1 from SIC13) and two from Calabria (1 from CAL08 and 1 from CAL09). No clear separation by region or putative morphological species is visible. Only the three populations from Sardinia (SAR14, SAR16, SAR17) which represent three different morphological species are separated from the other populations at K = 4, SAR16 is separated from all other populations at K = 8 (Fig. 5).

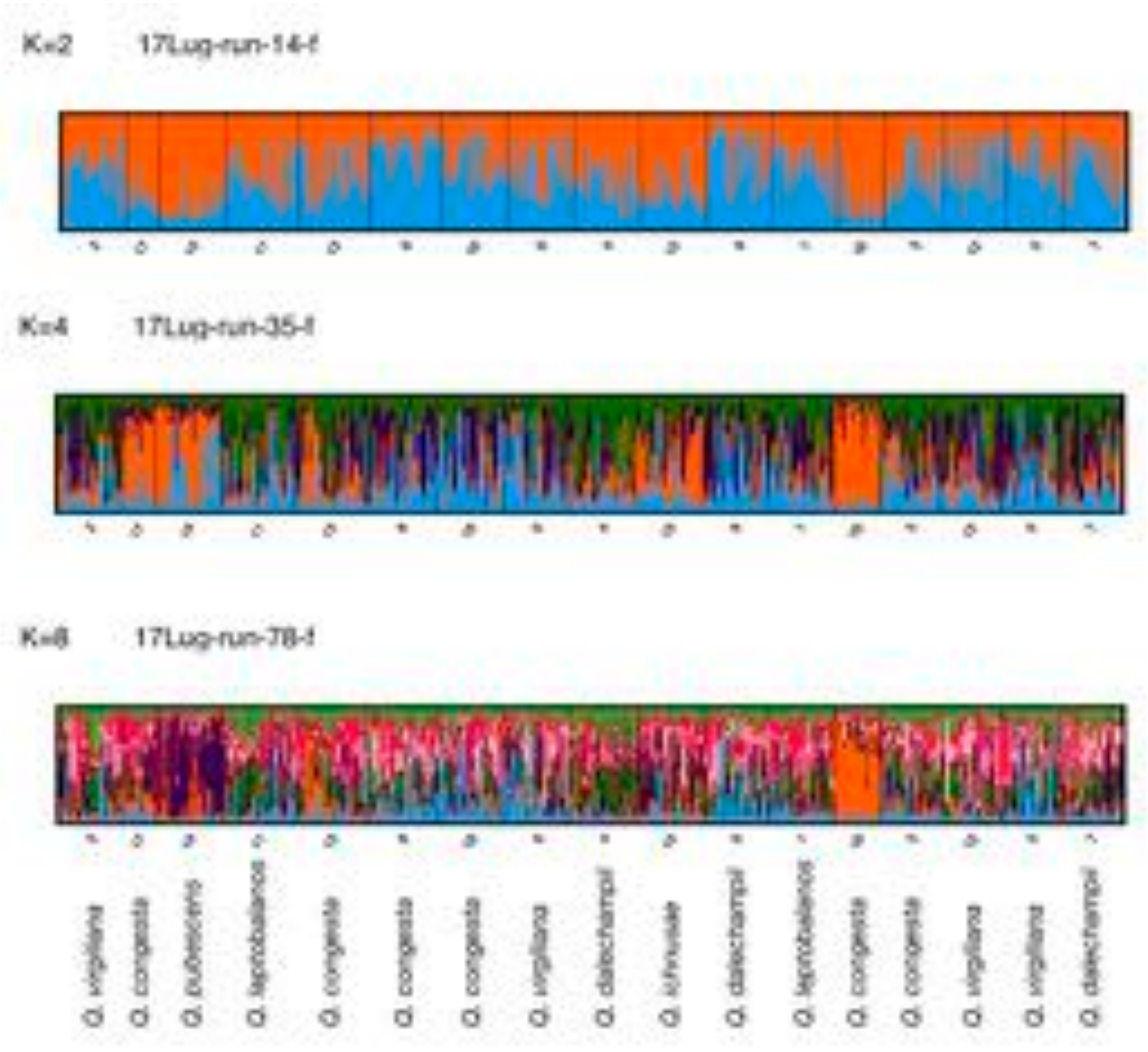

Fig. 5 STRUCTURE analysis (K=2, 4, 8) for all 17 population sampled in Calabria Sardinia and Sicily. K=8 resulted the most likely number of clusters.

Discussion

In the last twenty-five years we have witnessed an increase in molecular studies in Europe aimed at identifying possible distinctive characteristics within white oaks (Bacilieri et al. 1995; Bruschi et al. 2000; Csaikl et al. 2002; Bruschi et al. 2003; Curtu et al. 2007a, b; Fortini et al. 2009; Lepais et al. 2009; Lepais and Gerber 2011; Enescu et al. 2013; Yücedag and Gailing 2013; Fortini et al. 2015). However, no references were available about in-detail inter-population genetic studies undertaken for the southernmost part of Italy, Sicily and Sardinia although all these areas are unanimously considered of great importance for the evolution and phenotypic differentiation of white oaks. In fact, some interesting phylogeographic studies based on cpDNA diversity (Fineschi et al. 2004) had already shown that some of the haplotypes that colonized the European continent at the end of the last glacial maximum originated in Italy and in particular in the southern and island regions (Fineschi et al. 2002, 2004; Petit et al. 2002a). While it is true that cpDNA markers can be useful to establish the conformity of a given material to the populations of its origin and to trace possible routes of migration at a broad geographic scale, they provide no information on the systematic status of species living in sympatric conditions (see Curtu et al. 2007; Neophytou and Michiels 2013; Blanc-Jolivet and Liesebach 2015). In contrast, co-dominant markers, such as microsatellites, have successfully been tested to study genetic structures and distinguish oak species at regional or local scale (Degen et al. 1999; Gömöry 2000; Gugerli et al. 2007; Guicoux et al. 2011b; Hoeltken et al. 2012). Only recently, population genetic studies based on co-dominant nuclear markers have been carried out in white oak populations from restricted areas of southern Italy (Antonecchia et al. 2015; Fortini et al. 2015; Di Pietro et al. 2016, 2020). These studies were based on sampling protocols, which provided a high number of reference samples per population and a high number of populations per unit area.

The present study, in addition of being aimed at filling a gap in the genetic knowledge of the genus *Quercus* in the national territory, was fueled by the fact that the highest degree of phenotypic and taxonomic diversity among pubescent white oaks in Europe is described for southern Italy (see Tutin et al. 1993; Pignatti et al. 2017-2019). However, there is still a very lively debate, especially among the taxonomists of southern Europe, about the possibility of keeping all these 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 *Q. pubescens* Willd.). Accordingly, in addition to shedding light on genetic diversity of white oaks in an area still without detailed molecular studies, the aim of this work was to evaluate whether this high phenotypic and taxonomic diversity corresponded to an equally significant level of genetic diversity or geographical autonomy.

The populations investigated all show a fair level of genetic polymorphism. Taking into account individual loci, we have found an average number of alleles per locus rather high (14.08) and a variability per locus ranging between 8 (PIE227) and 20 (PIE102). The average number of alleles per population and locus was found to range between 2.9 (PIE227) and 11.5 (PIE 152). For H_o and H_e we found values of 0.649 and 0.683, respectively. All these values appear to be quite high if compared with those obtained in a similar study performed in the Apulian Peninsula in the south-easternmost sector of Italy (Di Pietro et al. 2020 Table 2) and in Mount Vairano (southern/central Apennines) (Supplementary Table S2). These results were unexpected, especially considering that Sicily, Sardinia and southern Calabria exhibit a higher geographical isolation if compared with that of the Apulian Peninsula. In fact, the degree of gene polymorphism for the study area was expected to be lower than that from continental areas where, at least in theory, it is conceivable that there may be a greater possibility of gene flow among populations. It is possible that the physiographical and geomorphological features of these two areas played a role in determining such an unexpected genetic pattern. The Apulian Peninsula is composed of carbonate plateaus (1116 m. the highest culmination) separated from the rest of the Italian Peninsula by a vast cultivated plain where the forest stands are scattered in a general matrix of olive groves, vineyards and wheat fields or separated from each other by mosaics of Mediterranean maquis and steppe-like grasslands (Biondi et al. 2010; Di Pietro and

Misano 2009). On the other hand, Sicily, Sardinia and southern Calabria are all characterised by remarkable mountainous systems whose highest peaks are all ranging between 1600 and 2000 m (see Aspromonte, Nebrodi, Madonie Gennargentu, Supramonte) with Etna volcano main culminations well above 3000 m. The presence of these mountains has probably made available a greater variety of habitats (and consequently of refuge sites) for the forest vegetation during the climatic oscillations of the Quaternary that allowed a greater territorial contiguity for the surviving oak woods. Three loci (PIE020, PIE239 and PIE242) showed an excess of homozygosity (deviation from Hardy-Weinberg equilibrium $P< 0.05$ for the first two loci and $p<0.01$ for the third). For PIE020, PIE239 null alleles were also detected and therefore possible non-random distribution of genotypes and distorted values of heterozygosity (Brown et al. 2005).

The level of intra-population genetic diversity in the study area shows an average value of alleles per population of 7.9 with an average allelic richness of 6.7. H_o and H_e values are also quite high (0.64 and 0.68, respectively) and do not show substantial differences if we consider the three study areas (Sicily, Southern Calabria and Sardinia) separately (Table 4). Instead, the Gst value is very different if the three study areas are compared. It is possible, however, that the high values shown by Sicily when compared to Calabria and Sardinia are influenced by the number of samples analyzed which for Sicily are more than double those of the other two regions. In general, the genetic diversity indices that emerge from this study were found to be significantly higher than those exhibited by the pubescent oak populations of the Apulian Peninsula but lower if compared with those found for a pubescent oak population from the southern/central Apennines (Mount Vairano) which were analyzed at the same markers (Supplementary Table S3). It is possible that the higher indices found at Mount Vairano site are linked to the fact that the *Q. pubescens* populations occurring there comprise other white oak species in the same forest community (e.g. *Q. frainetto* and *Q. petraea*) and are located in spatial contiguity with *Q. robur* stands of the foothills.

Genetic assignment, Principal Coordinate Analysis and the Neighbor-Joining tree separated Sardinian populations from Sicilian and Calabrian populations. This separation into two groups is probably to be addressed to the greater insularity of Sardinia as compared to Sicily, the latter being separated from southern Calabria by only a narrow stretch of sea (3 km). Despite the close geographical proximity between Calabria and Sicily, however, the interactions between the oak populations of these two territories may have been less than one might expect. In a study on the non-coding regions of chloroplast DNA of Italian populations of deciduous oaks, Fineschi et al. (2004) hypothesized that the missing seed migration from Calabria to Sicily of an eastern haplotype was related to the depth of the Ionian Sea which prevented its freezing even during the phases of maximum glacial extension and prevented the establishment of a land corridor between the two regions. However, not all authors agree on this point. According to some paleontologists during the Quaternary period territorial connections were established between Calabria and Sicily through which several mammalian taxa from continental areas dispersed into Sicily (Bonfiglio et al. 2002).

The correlation between genetic and geographical distance among populations expressed by the Mantel test was found to be positive and statistically significant, however very low. In fact, most of the genetic diversity found is observed within single individuals (92.05%) followed by genetic diversity among individuals within the same populations (5.15%) and among different populations (2.80%).

On the basis of Bayesian cluster analysis (STRUCTURE), the most probable number of clusters considering all individuals from all the populations is eight (K=8), followed by K=2 and K=4.

However, all the individuals sampled show a membership value which is not sufficient to assign them to any of these eight, two or four clusters. If we consider K=8 (highest likelihood), it emerges that the majority of the tree individuals exhibits a membership value which is about the same for all the eight clusters inferred by STRUCTURE. Only populations ST14, ST16 and ST14 presenting three different species in Calabria are separated from the remaining populations.

The result of the cluster analysis only partially mirrors that of PCoA. Also in the Neighbor-joining tree, the four Sardinian populations form a distinct cluster (C1) with a 51% bootstrap support and are separated from the rest of the oak populations. According to the phytosociological literature, these four Sardinian populations are assigned to at least three different species. Overall, the dendrogram does not cluster populations according to putative species, but shows a weak phylogeographic pattern with the Sardinian populations separated and populations collected in neighboring sites grouping together (see subgroups A1 and C2). Both the first and the second level of clustering, bring together different pubescent-oak (putative) species which, at least based on their original diagnosis and current coenological knowledge (Brullo and Marcenò 1985; Brullo et al. 1999; Mossa et al. 1999, Bacchetta et al. 2009) would have a very different ecology from each other. In the group of Sardinian populations *Q. congesta, Q. virgiliana* and *Q. ichnusae* group together. The Calabrian group includes *Q. congesta,* and *Q. dalechampii*. However, the two further subgroups of which the main Calabrian subgroup is composed of (CAL09-CAL10 and CAL07-CAL08) are both composed of a population of *Q. congesta* and one of *Q. dalechampii*. In particular, CAL09 is a *Q. dalechampii* population of the Meso-thermo Mediterranean bioclimate of the Gioia Tauro plain less than 100 meters a.s.l. while CAL10 is a *Q. congesta* population of the lower mountain belt of the Aspromonte massif at about 1000 m a.s.l. Only the subgroup SIC05-SIC06 clusters two populations belonging to the same putative species (*Q. virgiliana*).

The most plausible interpretation of the results is that all the oak populations sampled belong to a single oak taxon that is characterised by a large ecological and morphological amplitude and high genetic variability. Although there is still no scientific certainty, the morphological and molecular pattern among pubescent white oaks evidenced in this paper, and those already shown in previous papers for other pubescent-oak populations from the central Mediterranean area (Franjic et al. 2006; Viscosi et al. 2009, 2012; Ballian et al. 2010; Di Pietro et al. 2016, 2020), increasingly reinforce the idea that this "single highly variable pubescent oak taxon" could be the result of repeated events of hybridisation and introgression between an ancient pubescent white oak species (which for simplicity we could here name *Q. pubescens*) and other European white oak species (e.g. *Q. petraea, Q. frainetto, Q. robur*). These events would have taken place continuously since the Tertiary and may have even intensified during the Pleistocene following the drastic paleogeographic and paleoclimatic events that characterised this Era. Such a consideration, if translated into a taxonomic key, would exclude a too divisive classification within the collective group of *Q. pubescens*, and indeed would support the "minimalist" view considering just a single pubescent oak taxon at the rank of species. The present paper, however, also shows that the genetic patterns of the Sardinian populations are affected by the effects of geographical isolation, which is generating genetic diversification. This result is not unexpected if we consider that Fineschi et al. (2004) identified a Sardinian-Corsican endemic haplotype for oaks which distribution was restricted to these two islands. Actually, population SAR16 (a relic population composed of individuals currently identified as *Q. virgiliana/Q. amplifolia* from a northwestern Sardinian plain) exhibits comparatively low allelic diversity (see Table 4) as reflected in the complete absence of private alleles and the about lowest values for allelic richness and number of alleles among all populations. The comparatively low allelic diversity could be the result of geographic and topographic isolation. This population appears to be composed of few secular individuals very similar to each other, surrounded by several very young juvenile trees. This suggests that it may have experienced selective removal of trees aimed at favoring particular phenotypes that in addition to changing physiognomy and structure could have also influenced the genetic diversity of the forest ecosystem. A narrow selection of seed-producing trees may in fact lead to a lower variability in forest stands (Dostálek et al. 2011) so that it could be assumed that the secular individuals scattered in the SAR16 population (or at least a part of them) are none other than the progeny of few progenitor oaks.

Conclusion

As a first study on the genetic diversity of the southern Italy and major islands pubescent-oak populations, this paper displayed surprisingly high values for all the parameters linked to genetic diversity although more than two thirds of the study area was made up of island territory. We hypothesise that the rugged morphology and wide altitudinal amplitude may have played a role in preserving the spatial contiguity between oak woods in the study area during the Quaternary climatic oscillations and therefore in preserving also high levels of gene flow.

A genetic confirmation for a taxonomical classification providing up to seven pubescent oak species as occurring in the study area did not emerge from this study, despite reported by the most recent floras and checklist and by phytosociological papers as well. Such a result was not entirely unexpected when we consider that morphological and molecular analyses carried out on pubescent oak populations in south-eastern Italy (Di Pietro et al. 2016, 2020) demonstrated that neither morphological nor molecular results supported the occurrence of more than one pubescent oak species whereas four species were reported by previous phytosociological studies (Biondi et al. 2004, 2010). The oak material analysed in our study did not show a degree of molecular diversity, within and among populations, sufficient to support this wide taxonomical splitting. On the contrary, our results suggest to consider all the populations investigated as belonging to a single taxon which is characterised by a wide range of intra-individual and intraspecific genotypic and phenotypic diversity as result of ecological pressures to which particular groups of oak species are subjected (Kremer and Hipp 2019). In our opinion, especially in southern Italy, it has deeper roots. Roots involving innumerable events of hybridisation and introgression that could have happened between an ancestral pubescent oak (which for simplicity we will call here *Q. pubescens* s.l.) and other sympatric thermophilous white oaks over the ages. Thanks to the favourable geographical location of southern Italy, in Sicily and Sardinia these events could have occurred without significant interruptions even during the colder periods of the Quaternary where the different oak species (*Q. pubescens* s.l., *Q. petraea, Q. robur* and *Q. frainetto*) were forced to live in very restricted areas. Possible hints of a process of speciation in progress for the Sardinian populations related to the highlighted (weak) correspondence between genetic and geographic distance and to the geographical isolation of this island are premature and will require further and more detailed studies.

Beyond the phylogenetic or taxonomic relevance, the results have implications for Forest economy (timber certification) or nature conservation, if we consider that some of the oak names in issue occur in the list of diagnostic species for European Habitats included in the 92/43/EC Directive (European Commission 2013).

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SM4. Does the genetic diversity among pubescent white oaks in southern Italy, Sicily and Sardinia islands support the current taxonomic classification?

Di Pietro R.[1], Conte A.L.[2], Di Marzio P.[2], Fortini P.[2], Farris E.[3], Gianguzzi L.[4], Müller M.[5], Rosati L.[6], Spampinato G.[7], Gailing O.[5]

[1]Department PDTA, University of Rome Sapienza, I-00196 Rome, Italy
[2]Department Bioscience and Territory, University of Molise, I-86090 Pesche (IS), Italy
[3]Department of Science and Territory, University of Sassari, I-07100 Sassari, Italy
[4]Department of Agricultural and Forest Sciences, Università degli Studi di Palermo, I-90123 Palermo, Italy
[5]Faculty of Forest Sciences and Forest Ecology, Forest Genetics and Forest Tree Breeding, University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany
[6]School of Agricultural, Forestry and Environmental Sciences, University of Basilicata, I-8500 Potenza, Italy
[7]Department of Agriculture, Mediterranean University of Reggio Calabria I- 89122 Reggio Calabria, Italy

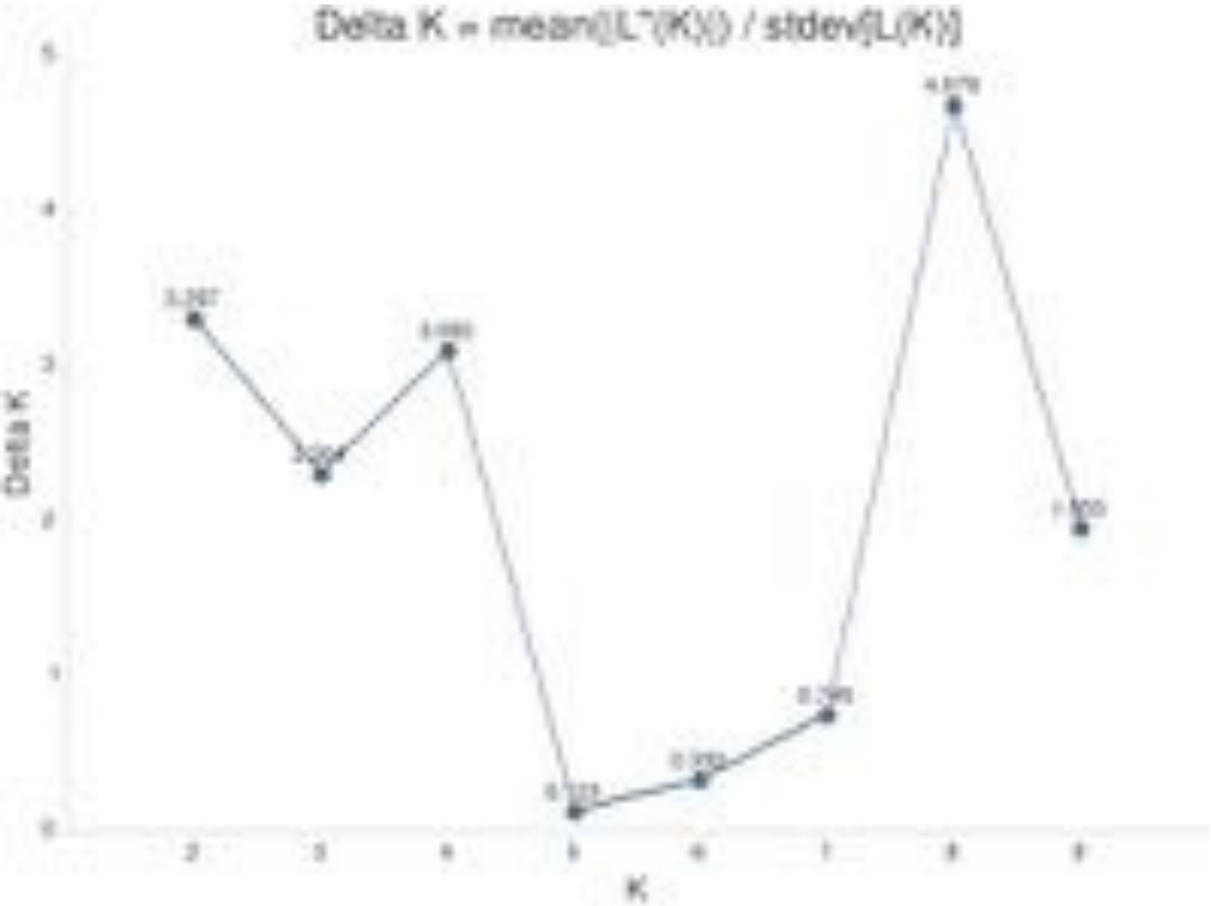
🖂 Corresponding author, Paola Fortini: fortini@unimol.it

Supplementary Table S1 Pairwise population matrix of G_{ST} values

	SIC01	**SIC02**	**SIC03**	**SIC04**	**SIC05**	**SIC06**	**CAL07**	**CAL08**	**CAL09**	**CAL10**	**SIC11**	**SIC12**	**SIC13**	**SAR14**	**SAR15**	**SAR16**	**SAR17**
SIC01		0.048	0.007	0.004	0.005	0.014	0.013	0.010	0.001	0.001	0.033	0.004	0.057	0.001	0.001	0.001	0.001
SIC02	**0.004**		0.003	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.004	0.001	0.001	0.001	0.001	0.001
SIC03	**0.007**	**0.007**		0.002	0.001	0.004	0.001	0.001	0.001	0.002	0.002	0.002	0.059	0.001	0.001	0.001	0.001
SIC04	**0.006**	**0.011**	**0.008**		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
SIC05	**0.007**	**0.012**	**0.010**	**0.014**		0.292	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
SIC06	**0.005**	**0.010**	**0.007**	**0.011**	0.001		0.023	0.001	0.001	0.009	0.002	0.001	0.003	0.001	0.001	0.001	0.001
CAL07	**0.006**	**0.010**	**0.008**	**0.013**	**0.010**	**0.004**		0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
CAL08	**0.005**	**0.007**	**0.012**	**0.016**	**0.010**	**0.008**	**0.007**		0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
CAL09	**0.010**	**0.010**	**0.011**	**0.018**	**0.008**	**0.008**	**0.008**	**0.006**		0.019	0.001	0.001	0.001	0.001	0.001	0.001	0.001
CAL10	**0.011**	**0.012**	**0.008**	**0.011**	**0.013**	**0.005**	**0.012**	**0.009**	**0.005**		0.001	0.001	0.001	0.001	0.001	0.001	0.001
SIC11	**0.004**	**0.012**	**0.008**	**0.012**	**0.008**	**0.007**	**0.010**	**0.014**	**0.011**	**0.014**		0.001	0.010	0.001	0.001	0.001	0.001
SIC12	**0.008**	**0.008**	**0.010**	**0.017**	**0.015**	**0.013**	**0.013**	**0.012**	**0.011**	**0.017**	**0.018**		0.001	0.001	0.001	0.001	0.001
SIC13	0.003	**0.009**	0.003	**0.010**	**0.013**	**0.006**	**0.009**	**0.012**	**0.011**	**0.011**	**0.005**	**0.011**		0.001	0.001	0.001	0.001
SAR14	**0.021**	**0.027**	**0.023**	**0.025**	**0.029**	**0.019**	**0.023**	**0.029**	**0.029**	**0.020**	**0.022**	**0.037**	**0.022**		0.001	0.001	0.001
SAR15	**0.010**	**0.014**	**0.007**	**0.016**	**0.015**	**0.011**	**0.008**	**0.017**	**0.017**	**0.016**	**0.009**	**0.020**	**0.011**	**0.013**		0.001	0.001
SAR16	**0.037**	**0.035**	**0.033**	**0.045**	**0.048**	**0.038**	**0.036**	**0.045**	**0.049**	**0.042**	**0.038**	**0.048**	**0.027**	**0.039**	**0.030**		0.001
SAR17	**0.023**	**0.023**	**0.016**	**0.029**	**0.026**	**0.015**	**0.021**	**0.029**	**0.030**	**0.017**	**0.024**	**0.034**	**0.018**	**0.023**	**0.015**	**0.029**	

G_{ST} values below the diagonal; significant values are in bold. Probability, based on 999 permutations, is shown above diagonal.

G_{ST} = analog of F_{ST} adjusted for bias: $G_{ST} = (cH_t-cH_s)/cH_t$.



Supplementary Figure S1 DeltaK graph plot by CLUMPACK. Optimal K by Evanno method is 8.

Supplementary Table S.2 Sample size and mean genetic diversity indices for the *Quercus pubescens* s.l. stands in Apulia (APU; Di Pietro et al. 2020), M. Vairano, Molise (MOL; Antonecchia G. unpublished data), Calabria (CAL), Sardinia (SAR), and Sicily (SIC)

Locus	Region	N	K	H_o	H_e	F_{IS}	Locus	Region	N	K	H_o	H_e	F_{IS}
PIE020	APU	312	7	0.583	0.585	-0.067	PIE239	APU	302	9	0.248	0.435	0.399*
	MOL	55	7	0.527	0.467	-0.129°		MOL	54	13	0.741	0.800	0.074°
	CAL	118	8	0.473	0.467	-0.012		CAL	115	11	0.575	0.645	0.109
	SAR	95	9	0.635	0.573	-0.107		SAR	86	3	0.239	0.207	-0.153
	SIC	261	13	0.490	0.467	-0.049		SIC	255	11	0.501	0.632	0.206
PIE102	APU	321	13	0.523	0.750	0.279*	PIE242	APU	318	14	0.748	0.841	0.093*
	MOL	55	13	0.782	0.815	0.041°		MOL	55	11	0.891	0.851	-0.047°
	CAL	117	15	0.859	0.797	-0.077		CAL	118	11	0.798	0.787	-0.014
	SAR	95	15	0.749	0.722	-0.037		SAR	95	13	0.844	0.808	-0.044
	SIC	258	18	0.719	0.735	0.021		SIC	258	15	0.791	0.830	0.047
PIE152	APU	314	17	0.834	0.890	0.043*	PIE243	APU	282	10	0.688	0.683	-0.038
	MOL	53	14	0.868	0.893	0.028°		MOL	52	7	0.827	0.730	-0.133°
	CAL	117	13	0.828	0.860	0.037		CAL	118	8	0.599	0.606	0.012
	SAR	94	13	0.902	0.834	-0.081		SAR	95	10	0.676	0.705	0.042
	SIC	257	18	0.829	0.877	0.055		SIC	260	12	0.596	0.636	0.062
PIE215	APU	281	12	0.384	0.707	0.417*	PIE258	APU	-	-	-	-	-
	MOL	55	11	0.745	0.755	0.013°		MOL	-	-	-	-	-
	CAL	113	12	0.837	0.822	-0.018		CAL	118	13	0.666	0.850	0.216
	SAR	95	11	0.863	0.810	-0.064		SAR	95	14	0.810	0.827	0.020
	SIC	263	12	0.654	0.748	0.126		SIC	259	16	0.528	0.849	0.378
PIE223	APU	318	11	0.811	0.822	-0.002	PIE267	APU	313	12	0.425	0.667	0.334*
	MOL	55	9	0.818	0.819	0.001°		MOL	54	6	0.722	0.759	0.048°
	CAL	113	11	0.803	0.823	0.025		CAL	118	8	0.738	0.764	0.035
	SAR	95	11	0.857	0.833	-0.030		SAR	95	9	0.810	0.696	-0.163
	SIC	263	12	0.687	0.766	0.103		SIC	259	9	0.733	0.737	0.006
PIE227	APU	323	4	0.223	0.251	0.095*	PIE271	APU	311	12	0.949	0.843	-0.153
	MOL	55	4	0.182	0.201	0.096°		MOL	55	9	0.818	0.831	0.016°
	CAL	115	4	0.230	0.204	-0.128		CAL	118	11	0.848	0.836	-0.015
	SAR	88	1	0.011	0.011	-0.023		SAR	95	10	0.857	0.774	-0.107
	SIC	265	8	0.383	0.447	0.143		SIC	259	12	0.843	0.808	-0.043

N number of individuals, *K* number of alleles at the locus, H_o observed heterozygosity, H_e expected heterozygosity, F_{IS} Fixation index (* = $P < 0.05$), ° significance not available

Supplementary Table S.3 Sample size and mean value of the genetic diversity parameters for the *Quercus pubescens* s.l. stands in Apulia, M. Vairano (Molise), Calabria, Sardinia, and Sicily

Region	**M**	**N_t**	**N_a**	**A_r**	**H_o**	**H_e**	**F_{IS}**
Apulia	15	312	5.8	4.4	0.583	0.629	0.039
Molise	55	55	9.4	9.2	0.720	0.720	0.001
Calabria	30	119	8.2	6.8	0.639	0.698	0.102
Sardinia	24	96	7.1	6.3	0.662	0.637	-0.017
Sicily	29	265	7.9	6.7	0.659	0.695	0.069

M mean number of samples per population, N_t total number of samples for all population investigated, N_a number of alleles, A_r allelic richness, H_o observed heterozygosity, H_e expected heterozygosity, F_{IS} inbreeding coefficient

Introduction to the ‘Oak Decline’

In the last century, a growing reduction of forest cover in the world was observed. The main causes are addressed to bad management of the habitats and overexploitation of the forest ecosystems (Bastin et al., 2019). This widespread and severe conditions of disturbance are linked to anthropic activities and this generates a complex of events that influenced negatively not only the forest ecosystems but also the local socio-economic situation (Menezes-Silva et al., 2019). The destruction and reductions of the forests have contributed to phenomena such as global warming, land-grabbing, emission of greenhouse gasses and other natural disasters that are producing great environmental imbalances.

The oaks forests are one of the communities that are majorly subjected to human disturbance owing to they are located at middle latitudes, where also the largest urban and industrial agglomerations placed.

In addition to the human direct pressure, there is also the climate change and the consequent global ecological changes that are threatening the conservation of the oak forests, and this especially in the Mediterranean basin (Gentilesca et al., 2017). One of the most worrying effects linked at the climate changes at our latitude is the oak decline.

The oak decline is a mix of poor health condition involving oak forests all around the world and that causes the death of hundreds' hectares of woodlands. This syndrome manifests itself generally in the late spring and summer, with symptoms such as leaf yellowing, summer defoliation, the death of the terminal axis of the branches (died-back), secretion of dark exudates from the trunk, thinning of the crown, production of epicormic branches along the trunk. Episodes concerning this disease have been recorded in central Europe since the 18th century (González Alonso, 2008). Still, in the last 40th, many other events are occurred in central and southern Europe, mainly around the Mediterranean basin (Gentilesca et al., 2017).

According to some authors, the causes that trigger the oak decline, are linked to a complex of negative conditions that act simultaneously and that often lead to the definitive death of the oaks following the previous stage of decline. The most recent studies suggested that some of the principal factors that trigger the oaks decline are climatic anomalies, such as heat-waves and anomaly drought period, but also hydraulic failures, carbon deficiency, attack of defoliant insects and other pests (Ragazzi et al., 2000, Thomas 2002, Choat et al., 2012, Keča et al., 2016, Colangelo et al., 2017).

In the article below I have tried to rebuild the oak decline events occurred in southern Italy in summer 2017, based on an ecological approach.

Chapter 5. Oak decline in the Mediterranean basin: a study case from the southern Apennines (Italy)[14]

Antonio Luca Conte[1], Romeo Di Pietro[2], Duilio Iamonico[2], Piera Di Marzio[1], Giuseppe Cilli[3], Donato Lucia[3], Paola Fortini[2].

[1]Department of Bioscience and Territory, University of Molise, 86090 Pesche (IS) Italy.
[2]Department PDTA, University of Rome Sapienza, 00196 Rome, Italy.
[3]School of Agricultural, Forestry and Environmental Sciences, University of Basilicata, I-8500

🖂 Corresponding author, A.L. Conte: conte.antonio79@gmail.com

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Oak decline in the Mediterranean basin: a study case from the southern Apennines (Italy)

A.L. Conte[1], R. Di Pietro[2], D. Iamonico[2], P. Di Marzio[1], G. Cillis[3], D. Lucia[3], P. Fortini[1]
[1]*Department Bioscience and Territory, University of Molise, I-86090 Pesche (IS), Italy.*
[2]*Department PDTA, University of Rome Sapienza, I-00196 Rome, Italy.*
[3]*School of Agricultural, Forestry and Environmental Sciences. University of Basilicata, I-85100 Potenza, Italy.*

Antonio Luca Conte https://orcid.org/0000-0002-5053-2561, Romeo Di Pietro https://orcid.org/0000-0003-4983-8931, Duilio Iamonico https://orcid.org/0000-0001-5491-7568, Piera Di Marzio https://orcid.org/0000-0002-3831-5388, Giuseppe Cillis https://orcid.org/0000-0002-1851-1533, Donato Lucia https://orcid.org/0000-0002-9321-4504, Paola Fortini https://orcid.org/0000-0003-4481-2126

Abstract
It is known that the decline of oaks forest can be triggered by an increase of climatic anomalies such as heat waves, droughts, and extreme cold. The present study aims to deepen the relationships between climate anomalies and oak decline basing on in-field observations made in the Lucanian Apennine (southern Italy) during summer 2017. Remote sensing was used to identify those areas affected by vegetation decline. A comparison of the climatic conditions recorded in 2015 and 2017 was carried out, these years being the hottest and the driest, respectively, since 1800. Satellite images and remote sensing data [Normalized Difference Water Index (NDWI), Temperature Condition Index (TCI)], and ground-based collected data [(Decline Severity (DES), Deficit/Surplus (D/S), Rainfall Anomaly Index (RAI), Standardized Precipitation Index (SPI)] were processed using GIS techniques to evaluate spatial distribution and time-scale evolution of the damage by oak decline. The results show that despite the heat peaks reached in 2015, it was not possible to highlight any clear sign of oak decline based on satellite images for that year. On the contrary, these signs were found to be evident by observing the satellite images of 2017 and confirmed by the Decline Severity assessment made in the field and further supported by NDWI and DNDWI indexes. Regarding possible factors that may have triggered the 2017' oak decline in the study area, it is not possible to provide a definite answer, at present. In this work it was hypothesized that an important role could have been played by the drastic reduction of rainfalls during the first semester of the year.

Key words: DES, Italy, Lucanian Apennine, NDWI, oak decline, Sentinel 2 (ESA).

Introduction

During the last forty years, the oak-decline phenomenon has affected the forests of the northern hemisphere, causing the death of hundreds of hectares of oak forest (Gottschalk & Wargo, 1997; Gentilesca *et al*., 2017). Although this has often been considered the result of local or regional episodic events, the interest in the oak decline has grown exponentially over time and, today, it is one of the most studied issues in the field of forest conservation (González-Alonso, 2008; Bussotti & Pollastrini, 2017; Hierro *et al*., 2017).

Oak decline is generally defined as a syndrome affecting individuals and populations of species belonging to *Quercus* genus, which manifests itself with clear signs of plant suffering, such as leaf yellowing, summer defoliation, death of the terminal axis of the branches (died-back), secretion of dark exudates from the trunk, thinning of the crown, and the production of epicormic branches along the trunk (Ciesla & Donaubauer, 1994; Ragazzi *et al*., 2000). Studies carried out on this topic over the past two decades have shown that the oak decline is to be related to the single or combined effects of many factors, such as climatic anomalies, hydraulic failures, carbon deficiency, and attack from defoliant insects and other pests (Manion, 1991; Ragazzi *et al*., 2000; Thomas *et al*., 2002; Choat *et al*., 2012; Keča *et al*., 2016; Colangelo *et al*., 2017). However, in recent years, much of the attention has focused on the climate and on rapid changes in temperature and rainfalls trends. Since 1950, many extreme climatic events have been recorded (IPCC, 2014) in various parts of the world, and, according to some worrying scenarios hypothesised for the near future (2020-2049), heat waves and periods of drought will happen with greater frequency and through progressively higher heat peaks (Allen *et al*., 2010; Mariotti *et al*., 2015; Lhotka *et al*., 2018).

In Europe, an increasing risk of drought moving along a W-E gradient is expected (Lindner *et al*., 2010). In the Mediterranean region, a decline in forest productivity is expected within a relatively short period of time due to a sharp increase in the length and intensity of drought periods and fire episodes (Lindner *et al*., 2010). In fact, a climatic deterioration has already

Corresponding author: Antonio Luca Conte. *Department Bioscience and Territory, University of Molise, I-86090 Pesche (IS), Italy; e-mail: conte.antonio79@gmail.com*

occurred since the end of the last century, in which the climate of the Mediterranean basin has shown a general tendency to become warmer and drier (Mariotti *et al*., 2015; Polade *et al*., 2017). Alongside, the beginning of the new millennium has been identified as a general period of Mediterranean forest decline related to drought (Di Filippo *et al*., 2010; Gentilesca *et al*., 2015).

Regarding the Italian Peninsula, climatic anomalies have occurred several times in the last twenty years with rainfalls far below the national average. Very low rainfalls, if compared to the rainfalls recorded during the period 1961-1990, were recorded in 2001, 2006, 2011, and, especially, in 2017 (Brunetti, 2017; Desiato *et al*., 2018; CNR-ISAC, 2019). On the other hand, very high temperatures have been recorded since 2000, with the high peaks in 2015 (Desiato *et al*., 2016-2018; CNR-ISAC 2019).

During the summer of 2017, large areas of the Lucanian Apennine (southern Italy) characterized by mixed oak woods exhibited an anomalous 'morphological status' with a yellow-brown colouring of the foliage and evident defoliation of the crown (Online Appendix I). All these symptoms are pertinent with the oak decline, which, among other things, had already been reported for several other areas of the Lucanian Apennines since the 1980s (Sicoli *et al*., 1992; Ragazzi *et al*., 2000; Gentilesca *et al*., 2015). Already then, it was assumed that anomalous climatic phenomena were responsible, although the relationships among the climatic and biotic factors involved in the decline phenomenon were not examined in depth.

It is certain, however, that the cases of oak decline have gone up during the past thirty years (González-Alonso, 2008; Gentilesca *et al*., 2015; Michel *et al*., 2018). Thus, it would be of primary importance to establish a shared methodological protocol of field and laboratory investigations for monitoring the spatial distribution and time-scale evolutions of the oak decline. This would certainly help to promote a global strategy of actions and policies to carry out effective management plans to preserve the functionality of forest ecosystems.

The aim of this study is to investigate the relationships between the climate anomalies that occurred in the last years in southern Italy and the oak decline phenomena observed in the Lucanian Apennine during summer 2017. The study was carried out combining detailed climatic data, remote sensing, and field surveys.

Materials and methods

Study area

The study area is located in the Lucanian Apennine, Potenza administrative province (~660,000 ha), western Basilicata; 15° 40' E, 40° 00' N (vertex SW), 16° 30' E, 41° 00' N (vertex NE). The Lucanian Apennine is part of Southern Apennine, which extends for about 120 km along an NW-SE direction in southern Italy. The lithological backbone is formed by calcareous rocks which are bordered, in the east side, by silicoclastic sediments and flyschoid deposits whereas the hilly areas at lower altitudes are characterised by conglomerates, and Plio-Pleistocene deposits (Patacca & Scandone, 2007). In this area, the oak forests mainly occur in the submontane and lower montane belts and are dominated by *Quercus cerris* L., *Q. pubescens* Willd. and *Q. frainetto* Ten. in the form of the following potential vegetation types: *Lathyro digitati-Quercetum cerridis*, *Lathyro jordani-Quercetum cerridis*, *Echinopo siculi-Quercetum frainetto*, *Centaureo centaurii-Quercetum pubescentis* (Di Pietro *et al*., 2010).

Satellite image interpretation (visible image) and GIS analysis

Satellite images in the visible band (spatial resolution: 10 × 10 m) for the periods March-October 2015 and March-October 2017 were examined (QGIS Development Team, 2018). These images were provided by the Sentinel 2 satellite, belonging to the European Space Agency's Copernicus monitoring system (ECMWF, 2018). The satellite data were available through the Climate Engine portal (Climate Engine, 2019; Huntington *et al*., 2017). The list of the parameters used in our analyses was reported in Table 1. The interpretation of satellite images allowed us to observe the spatial-temporal distribution of oak decline and select field survey areas (forest stands). To improve our dataset and avoid possible errors in the interpretation of the visible image (e.g. to consider the effects of recent fires or human activities on the vegetation cover as signs of oak decline episodes), we also analyzed the available satellite images from a longer period (2000-2017) taken from the Landsat 4/5/7/8 (NASA) satellites and RSDI Basilicata portal (Climate Engine, 2019; RSDI, 2019).

For the monitoring of the vegetation response to climate stresses the Normalized Difference Water Index (NDWI) was used. NDWI (Gao, 1996) is a satellite-derived index from Near-Infrared (NIR) and Short Wave Infrared (SWIR). This index allows removing variations induced by leaf internal structure and leaf dry matter content, improving the accuracy in retrieving vegetation water content (Ceccato *et al*., 2001; Wang *et al*., 2007). The NDWI values range between -0.4 and 0.4, where negative values represent oaks dying back due to the loss in leaf moisture content and positive values are considered a recovery of the leaves that grow back (see Wang *et al*., 2007). NDWI data were obtained for the period 2000-2017 by Landsat system and for the period 2015-2017 by Sentinel 2 system (Climate Engine, 2019) and they have been used to arrange a rectangular surface raster file including the whole study area. This file was converted to

Tab. 1 - List of data set used (Dat). Time series data available (Ts). Timescale resolution (Tr). Spatial scale resolution (Sr). Data supply (Das).

Parameter	Dat	Ts	Tr	Sr	Das
Rainfall	ALSIA Basilicata	> 2000	Monthly	Punctual	ALSIA Basilicata
Rainfall	CFD Basilicata	> 2001	Daily - Monthly	Punctual	Centro Funzionale Decentrato Basilicata
Temperature and Rainfall	Climatic Research Unit (CRU)	1901 - 2017	Monthly	0.5° x 0.5° or finer grid	Climatic Research Unit. University of East Anglia (www.cru.uea.ac.uk)
Visible Images	True colours. based on Red/Green/Blue bands	> 2015 Sentinel2 > 2000 Landsat	Daily	10 m 30 m	Sentinel 2 (ESA) and Landsat 4/5/7/8. Climate Hazard Group (https://clim-engine-development.appspot.com/fewsNet)
NDWI	NIR. SWIR	> 2015 Sentinel2 > 2000 Landsat	Daily	10 m 30 m	Sentinel 2 (ESA) and Landsat 4/5/7/8. Climate Hazard Group (https://clim-engine-development.appspot.com/fewsNet)
LST	MODIS	> 2000	8 days	1000 m	MODIS Terra 8-day. Climate Hazard Group (https://clim-engine-development.appspot.com/fewsNet)

a point-vector file using the GIS program ESRI ArcMap 10.1. Subsequently, the polygon-vector file of the borders of the forest stands was used to clip the NDWI's point-vector file. Finally, NDWI was used for mapping the died-back degree which followed the oak decline episodes in order to distinguish the areas where trees effectively died from those in which an active trend of tree canopy recovery was occurring. The differential NDWI (DNDWI) was calculated as $\mathrm{NDWI}_{2015}-\mathrm{NDWI}_{2017}$ (using Qgis Raster Calculator tool). Furthermore, we extracted all the NDWI point-vectors included in each stands area and on these, we performed statistics (mean, median, standard deviation, normal distribution).

The Temperature Condition Index (TCI) allowed the assessment of temperature stress conditions for vegetation (Kogan, 1995). TCI was calculated on the basis of the surface temperature (LST) datasets (Sun & Kafatos, 2007) from MODIS satellite (AVHRR thermal bands MODIS Terra 8 day, NASA) using the following formula: $\mathrm{TCI} = [(\mathrm{LST}max - \mathrm{LST}i) / (\mathrm{LST}max - \mathrm{LST}min)] \times 100$, where LST*i* is the last LST image available belonging to the period 24-31 August 2015 and 2017. While LST*min* is the last LST image available of the absolute minimum, LST*max* the last LST image available of the absolute maximum values. LST images were obtained from the Climate Engine Portal (Huntington *et al*., 2017; Magno *et al*., 2018).

Field survey

Field surveys were carried out at the end of summer 2017 only. The health status of oak's forest communities was defined using the Decline Severity scale (DES) modified from Mannerucci and Sicoli (2006), and Cullotta *et al*. (2016) (Tab. 2). We also assessed the defoliation of the crown by the oak decline's damage pattern: ‘tree-to-tree’ dieback (each adjacent individual is affected) or ‘salt and pepper’ dieback (several sick trees found in a healthy tree matrix) according to Ciesla and Donaubauer (1994). In addition, the phytosociological method (Braun-Blanquet, 1964) was used to identify the plant community type in issue.

Tab. 2 - Decline Severity damage observed scale (DES) used during summer 2017 field analyses. Dieback*: the condition of a tree that begins to die from the apex of its leaves towards the base of the branch due to an illness.

DES	Description of the symptoms observed
0	Healthy plants
1	Trees with crown damaged by dieback* for more than 20%
2	Trees with crown damaged by the dieback from 21 to 50%
3	Trees with crown damaged by dieback from 51 to 99%
4	Trees with 100% dieback with presence of epicormic shoots
5	Trees with crown and dead apical branches, secondary branches still alive with or without buds
6	Completely dead plant

Pluviometric data (D/S, RAI, and SPI)

Pluviometric data from 17 climatic stations were provided by the Decentralised Functional Centre of Basilicata (CFD Basilicata) and Lucanian Agency for Development and Innovation in Agriculture (ALSIA) (Online Appendix II). Pluviometric maps (isolines for general data and pluviometric indexes; D/S, RAI, and SPI, see below) were generated from a rainfall database (CSV file) interpolated with the ESRI ArcMap 10.1, using the Inverse Distance Weighted (IDW) method and fitting on the borders of the Potenza’s province using the contour tool. Owing to oak species having a growth response proportional to the amount of spring rainfall (Tessier *et al*., 1994; Corcuera *et al*., 2006; Di Filippo *et al*., 2010), we have used the first semester of each year as the pluviometric period of reference. Accordingly, we have arranged a further rainfalls dataset (using CRUTS 3.21; Online Appendix III) in which the monthly values and those summing the rainfalls of both the 1st and the 2nd semester of the year were shown (Harris *et al*., 2014 updated).

The Deficit/Surplus (D/S) is a measure of the difference between the rainfall value recorded during a given period (cumulative values) and the rainfall value recorded during a reference period (see e.g., Berterame *et al*., 2017). The D/S formula, which was calculated for each pluviometric station (ALSIA-CFD data set), is D/S = P_{ij} - P_{ik}, where P_{ij} is the cumulated rainfall of the *i*-semester of *j*-year and P_{ik} is the average of the average cumulative rainfall in the *i*-semester for the *k*-period.

The Rainfall Anomaly Index (RAI), proposed by Van Rooy (1965), is one of the most effective indexes to identify climatic anomalies (Keyantash & Dracup, 2002). The RAI values were obtained by comparing the first semesters of 2015 and 2017 (ALSIA-CFD data set) with an average of the 2001-2017 first semesters' series. The RAI was calculated using the equation $RAI = (R - \mu) / \sigma$, where R is the rainfall refers to the first semester of 2015 and 2017, μ is the long-term average rainfall (first semester of 2001 and 2017), and σ is the standard deviation. Low rainfalls values correspond to negative RAI values (Dutta *et al*., 2015).

The Standardised Precipitation Index (SPI) was used to identify and characterize drought periods (Mckee *et al*., 1993; Edwards, 1997) at different time scales (usually 1, 3, 6, 12, 24, and 48 months). In the present paper, SPI was computed on a time scale of 6 months, using the database of ALSIA and CFD Basilicata from 2001 to 2017. SPI values ranging from -1.0 to 1.0 reflect normal rainfalls. A dry regime (rainfall deficit) is indicated by SPI values lower than -1.0, whereas a wet regime (excess rainfall) by SPI values higher than 1.0.

Results

Satellite image interpretation (visible image) and GIS analysis

The satellite images interpretation made on an extended period (2000-2017) highlighted the occurrence of a severe episode of the oak decline for summer 2017. For this year (2017), satellite images allowed to identify 24 areas of the Lucanian Apennines in which the oak decline manifested itself clearly. Accordingly five forest stands (ST) which exhibited a situation of homogeneus oak decline were selected in order to analyze the monthly time evolution of this phenomenon (Fig. 1; Online Appendix IV). The results showed that the oak decline started during the first week of July in stands ST1, ST4, and ST5 and at the end of August in stands ST2 and ST3. The highest degree of damage for all the analysed stands was observed in the middle of September.

Normalised Difference Water Index (NDWI)

The NDWI analysis for the period 2000-2017 (Landsat dataset) showed that years 2000, 2001, 2008 and 2017 exhibited low NDWI values. However, the Sentinel 2 data, which were available for the period 2015-2017 only, showed that 2017 registered the lowest NDWI values (Tab. 3). In fact, during summer 2017 a widespread oak decline was found to be occurring in the whole study area with more than 500 hectares of

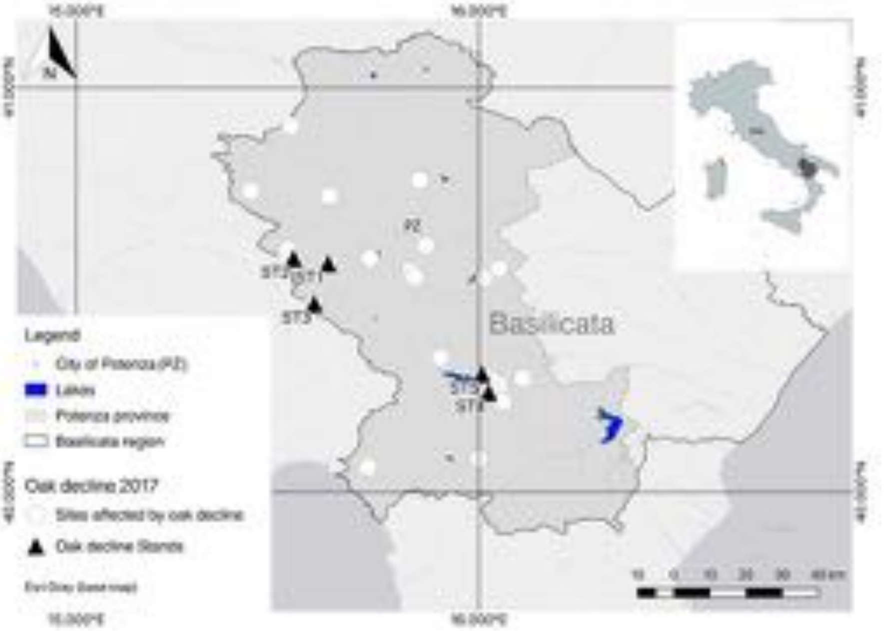

Fig. 1 - Map of the oak decline stands (ST1-5) subjected to the 2017 field investigations (see Online Appendix IV).

woods involved. The NDWI average values extracted from Sentinel 2 for the forest stands in issue (period 2015-2017) showed the following mean trend: 0.29 ±0.03 (2015), 0.30 ± 0.04 (2016), 0.21 ± 0.07 (2017).

The DNDWI ($NDWI_{2015}$–$NDWI_{2017}$) showed that although patches of various size of vegetation recovery were found over the Region, the large majority of the study area was characterized by extensive died-back patches (Fig. 2; Tab. 3).

Temperature Condition Index (TCI)

The TCI values recorded for 2015 and 2017 were found to be averagely similar, showing that the thermic stress suffered by the forest vegetation was severe on both dates. However, a different spatial distribution of TCI emerged comparing 2015 and 2017. In 2015 the TCI was uniformly widespread in the northern part of the study area, whereas, in 2017, a discountinous distribution was observed (Fig. 3). For this reason, in 2015, the stands ST1 and ST2 exhibited higher thermic stress (dark-red colour) than in 2017, with stress values ranging from 7.51% to 10.49% (vs. 14.59% ST1 to 15.02% ST2 in 2017). On the other hand, stations ST3, ST4, and ST5 exhibited similar TCI stress values (or slightly lower) in 2015 and 2017 (Tab. 4).

Vegetation response

The DES highlighted that the degree of crown's damage was classified as “4” in three stands (ST1, ST4, ST5), while in the remaining two stands (ST2, ST3), it was classified as “3” (according to the scale reported in Tab. 2). The ‘salt and pepper’ dieback pattern was detected in three out of the five stands (ST1, ST2, ST4) and the ‘tree-to-tree’ dieback in stands ST3 and ST5 (Tab. 5). The phytosociological sampling showed that ST1 and ST5 were dominated by *Q. pubescens* and that in ST3 *Q. pubescens* was co-dominant with *Q. cerris*. Differently in ST2 and ST4 the dominant oak species were found to be *Q. cerris* and *Q. frainetto* respectively (Tab. 5). During the field sampling, we observed that the forest decline appeared more marked at the woody edge of the forest stands. These edges were mainly composed of shrubby species (e.g. *Spartium junceum* L., *Cornus sanguinea* L., *Rosa canina* L. s.l., *Crataegus monogyna* Jacq., *Prunus spinosa* L., *Rubus* L. spp.) or medium-sized trees (*Acer mospessulanum* L., *A. campestre* L., *Pyrus pyraster* Burgsd., *Fraxinus ornus* L., and *Carpinus orientalis* Mill.).

Pluviometric data, D/S, RAI, and SPI

The rainfall data for the whole study area (Online Appendix III) showed that 2017 was the second driest year considering the period 2000-2017 and that, together with 2012, it was the only year showing rainfalls lower than 500 mm. If only the first semester was considered, then 2017 was found to be the driest year with only 213.75 mm of total rainfalls. A comparison with 2015 (the hottest year ever) showed that 2015 had yearly rainfalls about 46% higher than 2017. However, when the two semesters of the year were analyzed singularly, the total rainfalls of the first semester for 2015 (69.02 ± 46.15 mm) were found

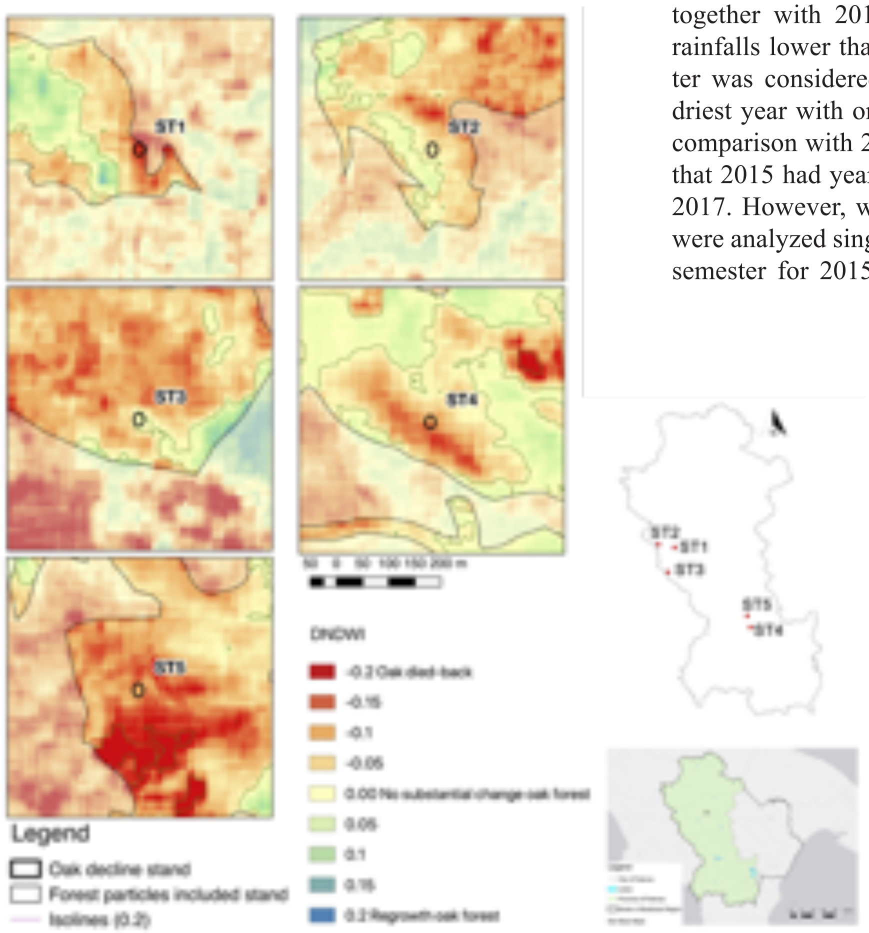

Fig. 2 - Details map of the stands affected by the oak decline (based on the Normalised Difference Water Index (DNDWI 2015-2017) = differential NDWI; $NDWI_{2015}$ $-NDWI_{2017}$ using Raster Calculator tool of Qgis). The decline areas are evidenced by the values under -0.05. For each stand reported in the small map in the lower right side of the figure (see Online Appendix IV) it is shown an enlargement of the wooded stands to highlight the degree of decline.

Tab. 3 - NDWI values for each stand based on the Landsat data set (time series 2000-2017) and Sentinel 2 (time series 2015-2017). SD = standard deviation. Data source Climate Engine. 2019. Desert Research Institute and University of Idaho. Accessed on (September 2019) http://climateengine.org

	Time scale 15 August to 17 September											
	LANDSAT 4/5/7/8											
Year	**ST1**	**SD**	**ST2**	**SD**	**ST3**	**SD**	**ST4**	**SD**	**ST5**	**SD**	**Annual Mean**	**SD**
Mean 2000	0.16	± 0.10	0.29	± 0.07	0.23	± 0.07	0.12	± 0.11	-0.04	± 0.01	0.15	± 0.12
Mean 2001	0.22	± 0.01	-0.03	± 0.03	-0.03	± 0.03	0.29	± 0.11	0.15	± 0.17	0.12	± 0.14
Mean 2008	0.23	± 0.20	0.27	± 0.23	0.22	± 0.18	0.21	± 0.10	0.15	± 0.15	0.22	± 0.04
Mean 2015	0.33	± 0.04	0.31	± 0.03	0.32	± 0.06	0.34	± 0.03	0.32	± 0.03	0.32	± 0.01
Mean 2016	0.29	± 0.01	0.36	± 0.03	0.37	± 0.02	0.30	± 0.00	0.29	± 0.01	0.32	± 0.04
Mean 2017	0.18	± 0.02	0.33	± 0.02	0.27	± 0.01	0.23	± 0.10	0.15	± 0.12	0.23	± 0.07
	SENTINEL 2											
Mean 2015	0.30	± 0.05	0.26	± 0.03	0.31	± 0.06	0.25	± 0.05	0.32	± 0.04	0.29	± 0.03
Mean 2016	0.30	± 0.04	0.30	± 0.04	0.37	± 0.06	0.28	± 0.08	0.26	± 0.06	0.30	± 0.04
Mean 2017	0.27	± 0.02	0.25	± 0.02	0.24	± 0.03	0.11	± 0.04	0.17	± 0.06	0.21	± 0.07

to be about twice those of 2017 (35.62 ± 30.55 mm), whereas the second semester displayed about similar rainfalls values for the two years.

The rainfall average of the ground-based collected data of first-semester (ALSIA-CFD data set) for the period 2001–2017 was 68.89 mm (± 16.40), and ranging from 34.55 mm (Genzano di Lucania locality, NE-Basilicata) to 132.78 mm (Nemoli locality, SW-Basilicata).

The lowest and the highest first-semester rainfall values during the 2001-2017 period occurred in 2008 (49.44 mm on average, ± 21.66) and 2009 (100.16 mm on average ± 45.50), respectively. In 2015, there was a surplus of rainfall of 28.03% compared to the average of the first semester of the 2001-2017 period (88.20 vs. 68.89 mm), whereas, in 2017, this rainfall average was 25.79% lower (51.12 vs. 68.89 mm).

Regarding the D/S index, a rainfall deficit occurred across in most of the Potenza administrative area during the first semester of 2017 (almost all values of D/S < 0, average to 17.77 mm ± 22.83), whereas no deficit occurred in 2015 (almost all D/S values were found to be > 0, average 19.31 mm ± 17.75). On the basis of the pluviometric maps generated (Fig. 4), the five stands were found to be characterized by positive values of D/S in 2015 and, therefore, by a surplus of precipitations, D/S values ranging from 24.43% for ST1

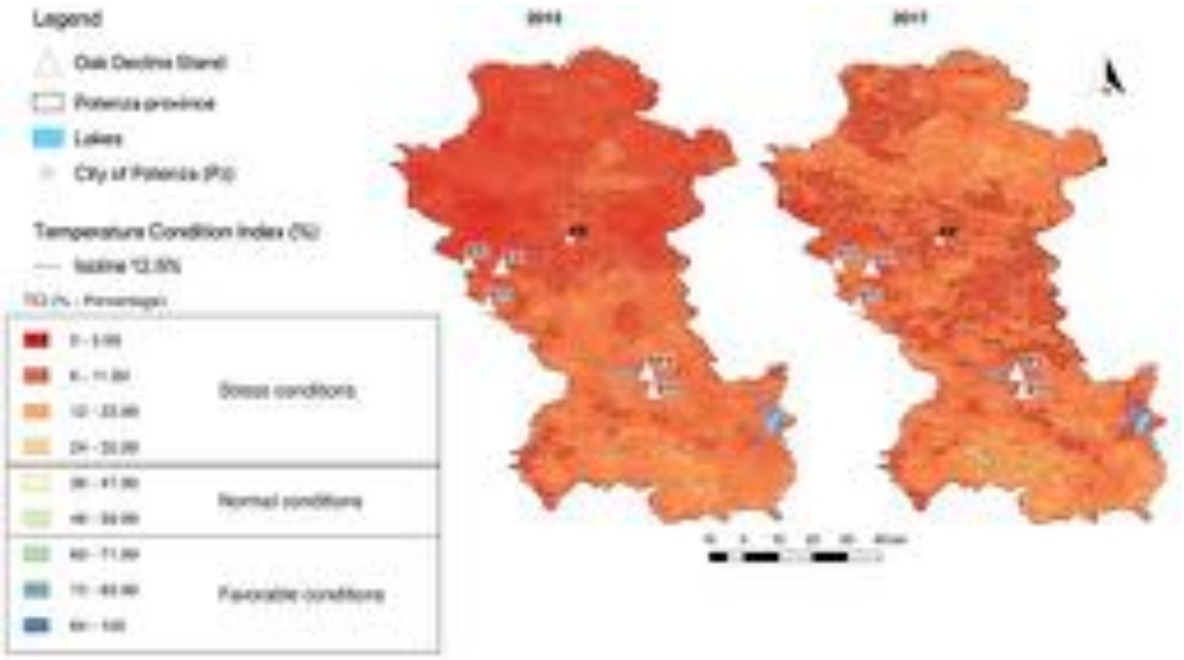

Fig. 3 - Distribution of the Temperature Condition Index (TCI) in summer 2015 and 2017.

to 27.38% of ST5, passing from ST2 with 26.31%, ST3 26.49%, and ST4 24.86%. In contrast, in 2017 these stands were characterised by negative values of D/S and, therefore, by a deficit of precipitations, D/S values ranging from -13.74% of ST5 to -20.34% for ST3 passing from -18.85% ST1, -18.58% ST2, and -17.13% ST4 (Tab. 4).

RAI values that referred to the first semester of 2017 were averagely lower when compared to those of the first semester of 2015 (Fig. 5). This result confirms that, in the first semester of 2017, the deficit was higher than during the same period in 2015 and of the average for the period 2001-2017. In 2017, the RAI values were mostly negative (14 out of 17 climatic stations), ranging from -0.44 to -1.61 (average -0.56 ± 0.66), whereas in 2015, almost all the RAI values were found to be positive and ranging from -0.16 and 1.84 (average 0.95 ± 0.65). The ST1 stand showed RAI = 0.78 for 2015, while both ST2 and ST3 RAI = 0.83. For both the stands located in the southernmost part of the study area (ST4 and ST5), the RAI 2015 values were found to be 0.80. In 2017 stands ST1, ST2, and ST3 exhibited low values of RAI (-0.75, -0.70, and -0.73, respectively), while stands ST4 and ST5 RAI of -0.53 and -0.44 respectively (Tab. 4; Online Appendix V).

Tab. 4 - Stand's dataset extrapolate from GIS. Percentage values of Deficit Surplus 2015 (D/S 2015 %). Percentage values of Deficit Surplus 2017 (D/S 2017 %). Values of Rainfall Anomaly Index 2015 (RAI 2015). Values of Rainfall Anomaly Index 2017 (RAI 2017). Percentage values of Temperature Condition Index 2015 (TCI 2015 %). Percentage values of Temperature Condition Index 2017 (TCI 2017 %).

Stand Code	D/S 2015 (%)	D/S 2017 (%)	RAI 2015	RAI 2017	TCI 2015 (%)	TCI 2017 (%)
ST1	24.43	-18.85	0.7817	-0.7531	10.4991	14.5951
ST2	26.31	-18.58	0.8371	-0.7003	7.5122	15.0245
ST3	26.49	-20.34	0.8312	-0.7303	15.2904	14.4737
ST4	24.86	-17.13	0.8049	-0.5323	15.3533	14.0824
ST5	27.38	-13.74	0.8049	-0.4497	15.3592	15.0756

SPI values for the year 2015 were found to be all positive, ranging from 0.202 to 1.881 (average 1.510 ± 0.533), whereas in 2017, these also exhibited negative values (up to -1.04) (Online Appendix V). On the basis of the Copernicus Europe an Drought Observatory drought scale (ECMWF, 2018), the precipitation regime in 2015 ranged from 'normal' to 'extremely wet' (most of the examined stations were included in the SPI categories 'moderately wet' and 'very wet'), whereas they ranged from 'moderately wet' to 'moderately dry' in 2017 (most of the examined stations are included in the 'normal' SPI category).

Discussion

In the present study, which concerned local episodes of oak forests decline in the Lucanian Apennines, we focused on the possible role of climatic anomalies in influencing this phenomenon.

We have found that the temperature anomalies recorded for 2015, the hottest year ever (CNR-ISAC, 2019), was not sufficient to trigger oak decline events. In fact, the year 2015, in addition to the high temperatures and possible heat waves, was characterized by a water surplus if compared to the average rainfalls calculated for the period 2000-2017 (as shown by D/S, RAI and SPI). It is therefore conceivable that precipitations that are not exceptional but at least not too deficient (especially in the first semester) have allowed the forest vegetation not to manifest an evident oak decline.

The oak decline observed during summer 2017 occurred concurrently to a general decrease in rainfall of about 20-30% compared to the period 2001-2017.

Tab. 5 - Vegetation features. Data collected on the field surveys in summer 2017 (*): Dominant species, Decline Severity (DES 2017), Pattern of decline, Vegetation series data.

Stand Code	Dominant species*	DES* 2017	Pattern of decline*	Vegetation series	Phytoclimate	Soil
ST1	*Q. pubescens*	4	Salt and pepper dieback	South-Appennines mesophilous neutro-subacidophilous (*Physospermo verticillati-Quercetum cerridis*)	Temperate oceanic semi-continental	Calcaric Cambisol, Calcaric Regosol
ST2	*Q. cerris*	3	Salt and pepper dieback	Centre-southern sub-Mediterranean and meso-Mediterranean Appennines neutro-basiphilous (*Roso sempervirentis-Quercetum pubescentis*)	Temperate oceanic semi-continental	Haplic Andosol Molli-Vitric Andosol Epilepti-Vitric Ando
ST3	*Q. cerris* - *Q. pubescens*	3	Tree to tree dieback	South-Appennines mesophilous neutro-subacidophilous (*Physospermo verticillati-Quercetum cerridis*)	Semi-continental oceanic transition	Haplic Andosol Molli-Vitric Andosol Epilepti-Vitric Ando
ST4	*Q. frainetto*	4	Salt and pepper dieback	South Appennines thermophilous neutro-subacidophilous (*Lathyro digitati-Quercetum cerridis*)	Temperate oceanic semi-continental	Calcaric Cambisol Skeleti-Calcaric Haplic Calcisol
ST5	*Q. pubescens*	4	Salt and pepper dieback	South-Appennines mesophilous neutro-subacidophilous (*Physospermo verticillati-Quercetum cerridis*)	Temperate Oceanic transition	Calcaric Cambisol, Calcaric Leptosol

This observation agrees with averages of the D/S and RAI, which were negative in 2017 (-17.77 mm and -0.56, respectively), whereas they were positive in 2015 (19.31 mm and 0.95, respectively). Also, the SPI index highlighted a difference in rainfall, which was up to extremely wet in 2015 and up to moderately dry in 2017. These evidences indicate that drought stress is probably the factor that is majorly involved in triggering oak decline more than temperature anomalies. However, it is not to be excluded that although extremely high temperatures are not sufficient, on their own, to trigger the oak decline, they may not have acted negatively in 2017 by widening the discomfort for a forest vegetation which was already exhausted by the water deficit. In fact, it should not be forgotten that, in addition to being the driest year since 1800, the year 2017 was also classified as the fourth warmest (CNR-ISAC, 2019). Our analyses regarding rainfall, carried out on both raw data and combined-indexes, revealed that a rainfall deficit occurred during the first semester of 2017 and it was absent during the same period of 2015. In addition, the temperature data did

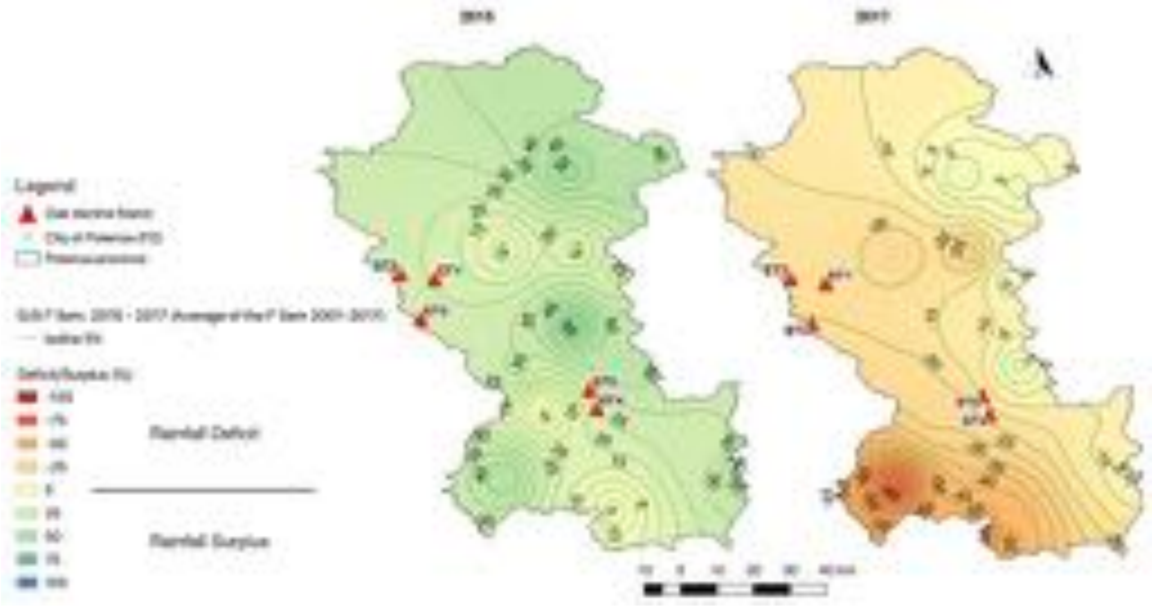

Fig. 4 - Deficit/Surplus (D/S) values for each Pluvio station in the 1st semester 2015 and 2017. D/S values reported in %. Pluviometric station code number (PS) in Online Appendix II.

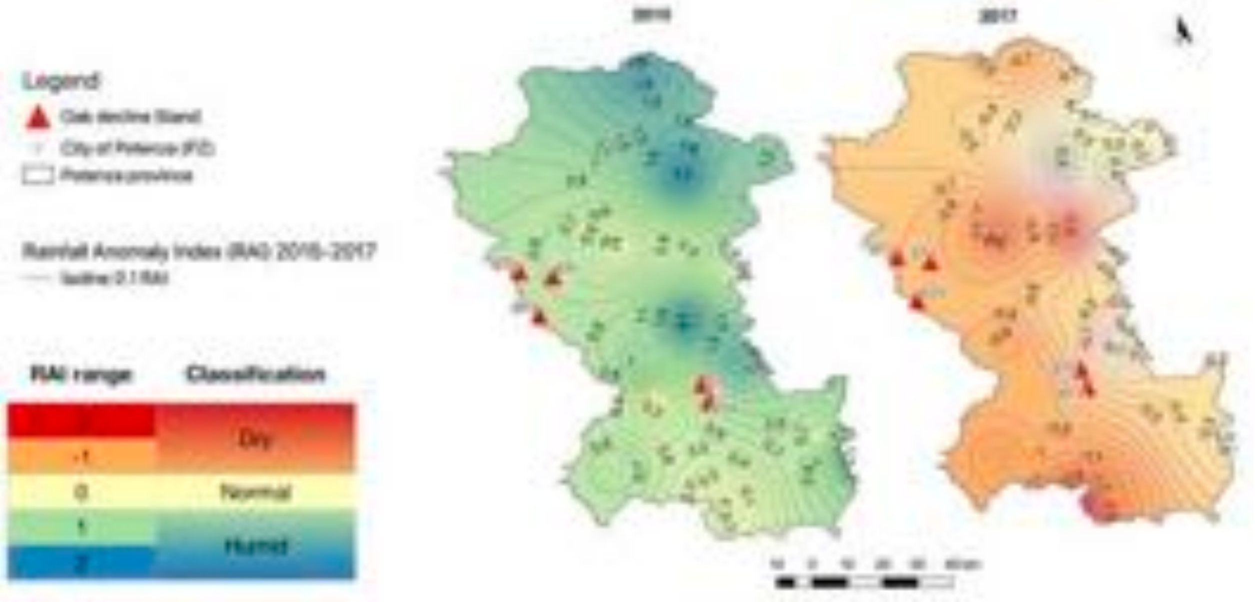

Fig. 5 - Rainfall Anomalies Index (RAI) in summer 2015 and 2017.

not provide significant differences between 2015 and 2017 in terms of thermic stress (TCI).

A worsening of the tree canopy conditions, which occurred passing from 2015 to 2017, was testified also by DNDWI and confirmed by DES. It is to be excluded any possible contribution of 2016 in determining the oak decline in 2017 being the NDWI values for 2016 even higher than those observed for 2015.

Vautard *et al*. (2007) observed that very hot summers (potentially able to trigger decline), are often preceded by dry winters. From what emerged in our work this statements cannot be confirmed or at least not completely. In fact, the correlation between hot summer and dry winters was pertinent with 2013, 2014 and 2017 and not pertinent with 2015 and 2016.

The fact that 2017 exhibited the lowest rainfall amount only for the first semester of the year would confirm that spring to early summer water availability is probably crucial in the seasonal cycle of the *Quercus* species and that climate anomalies occurring in this period could play a major role in triggering the oak forest decline. In broad terms, the oak decline could be viewed as a delayed response to spring drought conditions (see also Di Filippo *et al*., 2010; Natalini *et al*., 2016; Sánchez-Salguero *et al*., 2017) although according to other authors (see Gottschalk & Wargo 1997; Kabrick *et al*., 2008; Allen *et al*., 2010) drought, taken alone, would not be sufficient to cause decline and would require the action of other contributing factors such as heat stress, previous winter wind and frost damage, nutrient-deficiency and competition.

Concerning the distribution pattern of the oak decline, Mueller-Dombois (1992) argues that a possible cause of the decline is the synchronous senescence of the trees (called 'cohort senescence'), which would be able to spread rapidly from one area to its neighbouring ones. However, in the stands investigated, we have observed many dying trees occurring in a matrix of healthy trees, i.e. 'salt and pepper'.

Several authors (e.g., Camarero *et al*., 2016; Gentilesca *et al*., 2017; Colangelo *et al*., 2017) reported that the oak decline in mixed forests of the hilly and submontane belts of southern Italy involved *Q. cerris* more frequently than *Q. pubescens*. However, in our study area *Q. pubescens* would appear to be the most affected species (see Tab. 5). When the levels of drought stress become particularly severe, prolonged over time and possibly associated with other negative climatic anomalies concerning the winter or spring period, the fact that *Q. pubescens* woods tends to occupy the south-facing slopes, here as in the rest of the Apennines (see for example Ubaldi *et al*. 1984; Di Pietro & Blasi 1998; Allegrezza *et al*. 2003), makes them particularly exposed to undergo a more extensive and intense oak decline. As regards the observed evidence that the woody edges of the forest stands appeared to be more subjected to the negative effects of the decline we have not a definitive answer. As suggested by some authors (e.g., Van Gunst *et al*., 2016; Colangelo *et al*., 2017) it is possible that the shrub species forming the forest woody edges are more likely to die due to their inability to obtain water from great depths. At the same time it cannot be a-priori excluded that the woody edges acted more sensitively to oak decline, owing to the extremely severe drought conditions experienced by the ecotonal fringes when compared to those of the core areas where almost only dominant oak tree species are found.

Concluding remarks

The oak decline events in summer 2017 had a widespread effect throughout the Lucanian Apennine and involved more than 500 hectares of woods. The oak decline events observed in the field and assessed with DES were also confirmed by the NDWI datum. Our results suggest that the forest decline that we observed in 2017 in southern Italy could be majorly linked to the rainfall deficit that characterized the first semester of this year more than possible temperature anomalies.

The oak decline evidenced in the Lucanian Apennines is to be viewed as the local emergence of a phenomenon that is actually affecting the entire Mediterranean basin. The results obtained in our paper are pertinent with those published for other Mediterranean areas, where several other species of trees were involved. Similar cases of oak decline have recently been documented for some deciduous oak forests of central Italy (Castelporziano protected area in the Lazio Region), where the nefarious effects of the oak decline were found to be particularly significant in summer 2017 (Recanatesi *et al*., 2018). In other studies regarding the near East and Spain (Hosseini *et al*., 2017; Sánchez-Salguero *et al*., 2017) other species such as conifers (*Pinus halepensis* Mill., *P. nigra* J.F. Arnold), other deciduous (*Q. faginea* Lam.), semideciduous (*Q. brantii* var. *persica* (Jaub. & Spach) O.Schwarz), and evergreen oaks (*Q. ilex* L., *Q. suber* L.) were found to be involved in the oak decline phenomenon.

As already observed in the majority of the papers published over the last few years on the oak decline topic, the cause-effect relationships are still not clear and the role of the ecological factors involved not yet established.

A protocol of analyses, based on the combination of remote sensing techniques and field surveys would seem the only way forward although it will be probably necessary to implement the detail of the analysis carried out in the field. A new and shared sampling protocol could become an important tool in order to carry out oak decline risk maps at large scale useful for preserving the forest heritage in the whole Mediterranean area.

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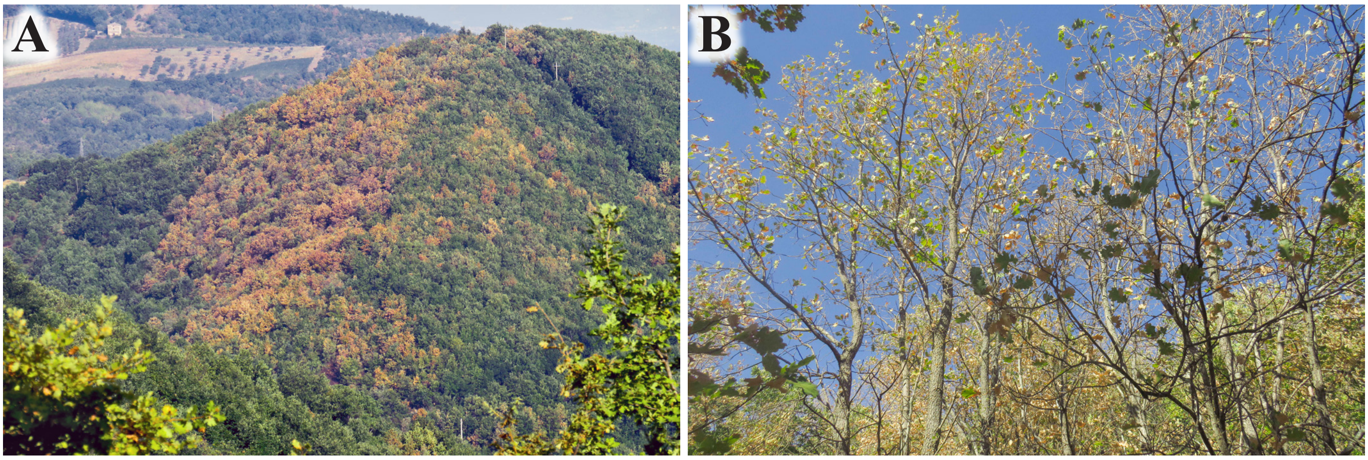
Appendix I

(A) The oak decline at the 2017-08-27. San Martino d'Agri (Pz). (B) Defoliation of trees at the 2017-09-17. Montemurro (Pz).



Appendix II

Climatic stations located in the administrative Provinces of Potenza and Matera. Pluviometric station code number (PS). Longitude and Latitude of DD UTM WGS84 (Data base from ALSIA and CFD Basilicata).

PS	Climate Station	Municipality	Altitude m asl	Longitude	Latitude
1	C.da Ripa D'api	Genzano di L. (PZ)	320	16.09416666	40.7991667
2	Loc. Abetina	Laurenzana (PZ)	938	15.96861111	40.4508333
3	Az. Bosco Galdo	Villa D'Agri (PZ)	595	15.82861111	40.3494444
4	C.da Pipoli	Acerenza (PZ)	420	15.95999999	40.8255556
5	Grassano scalo	Grassano (MT)	190	16.23638888	40.5969444
6	Piano delle Rose	S. Giorgio L.(MT)	455	16.38777777	40.1102778
7	C.da Montecrispo	Campomaggiore (PZ)	824	16.07138888	40.5688889
8	C.da Lupara	Guardia Perticara (PZ)	616	16.09833333	40.3627778
9	Az. Agr. Ventrona	Nemoli (PZ)	500	15.79666666	40.0750000
10	Loc. Serra del Ponte	Brindisi di Montagna (PZ)	820	15.97361111	40.6388889
11	C.da Torre	Stigliano (MT)	240	16.33416666	40.3950000
12	C. da Pedali	Viggianello (Pz)	616	16.06250000	39.9875000
13	AASD Pollino	Rotonda (Pz)	566	16.02222222	39.9491667
14	C. da Trutolo	Sarconi (Pz)	662	15.88944444	40.2488889
15	AASD Gaudiano	Lavello (Pz)	180	15.84861111	41.1016667
16	Piano delle Maniche	Senise (Pz)	270	16.30750000	40.1600000
17	Potenza	Potenza (Pz)	829	15.80153900	40.6369020

Appendix III

Rainfall data of the study area. Data source from Climatic Research Unit, University of East Anglia, CRU TS 3.21 (Data available on September 2019; https://www.globalclimatemonitor.org/).

	Rainfall of I Semester			**Rainfall of II Semester**			**Annual Rainfall**		
	Monthly average (mm)	**Standard Deviation**	**Total (mm)**	**Monthly average (mm)**	**Standard Deviation**	**Total (mm)**	**Monthly Average (mm)**	**Standard Deviation**	**Total (mm)**
2000	37.95	± 21.49	227.70	61.10	± 40.34	366.65	49.52	± 33.75	594.35
2001	77.80	± 49.28	466.80	30.25	± 33.57	181.50	54.02	± 47.86	648.30
2002	43.43	± 37.69	260.60	80.55	± 43.40	483.30	61.99	± 44.04	743.90
2003	55.90	± 40.83	335.40	90.90	± 77.07	545.45	73.40	± 62.91	880.85
2004	70.61	± 23.35	423.70	69.04	± 55.64	414.25	69.82	± 41.74	837.95
2005	66.29	± 37.34	397.75	97.18	± 65.06	583.10	81.73	± 54.22	980.85
2006	76.22	± 36.03	457.35	68.87	± 37.27	383.25	70.05	± 36.40	840.60
2007	70.30	± 29.70	421.85	68.90	± 49.85	413.40	69.60	± 40.14	835.25
2008	47.15	± 26.31	282.90	90.48	± 81.94	542.90	68.81	± 63.50	825.80
2009	107.53	± 73.46	645.20	68.11	± 59.20	408.70	87.82	± 68.28	1053.90
2010	73.65	± 26.95	441.90	81.49	± 75.94	488.95	77.57	± 55.87	930.85
2011	70.55	± 29.30	423.35	46.19	± 35.44	277.15	58.37	± 34.15	700.50
2012	46.00	± 41.73	276.00	28.87	± 26.67	173.25	37.43	± 35.35	449.25
2013	71.15	± 39.44	426.95	55.09	± 41.29	330.55	63.12	± 40.33	757.50
2014	58.07	± 27.28	348.45	42.57	± 23.47	255.45	50.32	± 26.11	603.90
2015	69.02	± 46.15	414.15	48.07	± 47.41	288.45	58.55	± 46.99	702.60
2016	69.15	± 27.73	414.90	55.78	± 42.12	334.70	62.46	± 35.54	749.60
2017	35.62	± 30.55	213.75	46.93	± 44.47	268.10	41.27	± 37.76	481.85
Mean	65.20	± 35.81		62.80	± 48.90		63.10	± 44.72	756.54
Standard Deviation	± 17.45			± 20.32			± 13.40		± 162.10

ID Points: 33624-33625. CRU TS 3.21 Data source:@Climatic Research Unit. University of East Anglia:CRU TS 3.21@NOAA:GHCN@DWD:GPCC made available under the ODL

Appendix IV

Characteristics of the five stands monitored with field survey.

Stand Code	Municipality and Province	Coordinates UTM-WGS84	Altitude m a.s.l.	Aspect (degrees)	Slope (%)	Stand’s Area m^2
ST1	Tito (Pz)	552979.00 E - 4490728.00 N	785	155°	43	400
ST2	Savoia di Lucania (Pz)	545712.00 E - 4492057.00 N	574	230°	67	400
ST3	Brienza (Pz)	550099.00 E - 4479755.00 N	958	180°	50	400
ST4	San Martino d’Agri (Pz)	587075.81 E - 4455612.61 N	756	170°	28	400
ST5	Montemurro (Pz)	585744.44 E - 4460624.09 N	843	230°	36	400

Appendix V

Standardised Precipitation Index (SPI). Deficit/Surplus (D/S) and Rainfall Anomaly Index (RAI) values for each Pluvio station in the 1st semester 2015 and 2017. PS = numbers of Pluvio stations in Online Appendix 2.

PS	SPI		D/S				RAI	
	2015	2017	2015 (mm)	2015 (%)	2017 (mm)	2017 (%)	RAI 15	RAI 17
1	1.751	0.674	15.91	44.44	6.51	18.18	1.210	0.495
2	1.405	-1.036	44.65	58.86	-12.25	-16.15	1.765	-0.484
3	1.341	0.305	23.24	31.20	-20.56	-27.62	1.057	-0.935
4	0.202	-0.524	25.59	51.08	8.69	17.35	1.843	0.626
5	1.881	-0.739	25.15	47.06	-13.95	-26.10	1.710	-0.949
6	2.326	1.175	23.71	42.73	-3.09	-5.57	1.386	-0.181
7	-	0.994	13.66	24.61	-2.34	-4.21	0.722	-0.124
8	1.405	-0.643	24.13	39.97	6.13	10.15	1.448	0.368
9	0.000	0.994	59.65	43.59	-73.45	-53.67	0.881	-1.084
10	0.000	0.000	7.66	12.14	-26.84	-42.50	0.351	-1.230
11	0.000	0.842	19.56	38.79	-2.24	-4.43	1.265	-0.145
12	1.881	0.332	-4.43	-4.80	-43.18	-46.79	-0.114	-1.115
13	1.881	0.253	14.39	13.33	-58.91	-54.59	0.395	-1.615
14	1.405	-0.050	-4.28	-5.07	-23.57	-27.90	-0.163	-0.900
15	-	0.025	19.71	39.12	-9.59	-19.03	1.613	-0.785
16	-	0.358	15.88	25.23	-9.32	-14.82	0.616	-0.361
17	1.126	-0.915	4.09	6.64	-24.11	-39.09	0.211	-1.242

Chapter 6. Synthesis and Outlook

To conclude this Doctor of Philosophy thesis, I will provide a summary of what were the aims of the paper and what have been the main achievements we've got and the points of discussion raised.

The first aim of the paper was that of trying to clarify the biosystematic and taxonomical position of a group of critical pubescent oak taxa, distributed mainly in the Italian islands and throughout central-southern Italy.

The interest of this work, however, cross the national borders being some of the investigated taxa (e.g. *Quercus virgiliana* and *Q. dalechampii*) currently listed in studies an in the national Floras of several southern European countries, such as Slovenia, Serbia, Croatia, Romania, Bulgaria, Greece, Hungaria, and the Czech Republic.

On the other hand, for two centuries (that means from the moment in which these species were described for the first time), these oaks became the centre of a complex taxonomic debate aimed, first, to find for them a consistent set of diagnostic traits with which justify their possible systematic autonomy, and second, to identify the proper taxonomical rank.

Over the years, dozens of researchers have dealt with this topic in various ways, each study provided new hypothesis and data in support of a possible new classification system. However, to date, we must take note that the taxonomical issues around the pubescent oaks have remained unresolved.

We could say that the results obtained till now do not meet current expectations in the systematic, biogeographical, coenological and conservation fields as well as unmistakably taxonomic. Obviously, a doctoral thesis cannot for its part offer the definitive solution to a complex problem, such as that of European white pubescent oaks.

Nevertheless, in my work, I have tried to provide new data and reliable results that add a contribution to the knowledge of this group. In particular, my work tried to construct a multidisciplinary framework around the pubescent oak group, including data from palaeogeography, palaeobotany, phylogenesis, taxonomy, genetics, morphology and ecology. This allowed me to investigate different aspects of the origin, evolution, current distribution and taxonomic issues linked to this group. Besides, I have carried out morphological (morphometry) and molecular investigations (EST-SSRs) which have provided tools and data to evaluate the divergence of some characters and genotypes in a wide range of 396 individuals, from 17 populations sampled in Sicily, Calabria and Sardinia.

The study area has clearly not been chosen by chance. In fact, in this area, is currently reported the maximum taxonomic diversification of pubescent oak at European level, with eight taxa recognised at the rank of species in the last edition of the Italian national flora (Pignatti et al., 2017).

Furthermore, always in this area, are located the '*loci classici*' of almost all the species investigated. The study of the oaks populations occurring in the proximity of these areas would have assumed crucial diagnostic importance in a not only taxonomic but also nomenclatural key.

In a nutshell, the question I asked myself in this doctoral thesis was the following:

- In light of current knowledge on the taxonomy and biosystematics of Italian pubescent oaks, what is it admissible to say with certainty and what has not been demonstrated yet?
- Is it possible to say that there are eight pubescent oak species in Italy?

- Is it possible to indicate in the phytosociological field that in Italy there are communities that we freely could identify as *Quercetum virgilianae, Quercetum dalechampii, Quercetum leptobalanae, Quercetum congestae* etc?
- Is there a correlation between the morphological diversity of the populations examined and their genetic diversity?

In my morphological survey, I did not find significant diagnostic traits that can justify the distinction between one or more group of individuals from another. There are significant dimensional characteristics, such as leaf area, perimeter, lobe depth ratio, lobe width ratio, etc., which do not have a taxonomic relevance to justify the recognition of species. Often, the variability of these traits is determined from the plasticity response to external ecological factors.

The results obtained from molecular analyses return quite similar to the one traced by the morphological results. Our data showed that the variability '*within individuals'* is higher than '*between individuals'* and '*between populations'*. Moreover, in this case, the Bayesian analysis (without prior assignment) does not report clear results, does not return homogeneous clusters. The hypothetical divisions in clusters obtained, K= 2, 4 or 8, are complex to interpret and present clusters with membership values (Q value) that are always very low. There is only one station (SAR16, see chapter 4) with three out of 480 samples that approach to a Q value > 0.90. This means that majority of individual shares a fraction of genotype similar to other individuals, but none of these genotypes is dominant; therefore, there is not a sufficient degree of genetic diversity as such to justify belonging to different taxonomic ranks.

In conclusion, I believe that the peculiar morphological differences (often local) indicated by some authors (Brullo et al., 1999; Pignatti et al., 2018) and accepted from other authors in Italy (Conti et al., 2005; Bartolucci et al., 2018) could be the result of a wide range of intra-individual and intraspecific phenotypic diversity.

Probably, this group can count on a basic genetic diversity, which is able to generate different phenotypic responses based on ecological pressures to which they are subjected (Kremer & Hipp, 2019; Hipp et al., 2019).

In my opinion, these phenotypic differences fall within a '*normal'* distribution (in statistic terms) of the phenotypic frequencies of a hypothetical single taxon that we could provisionally name *Q. pubescens* sensu lato.

Many studies (see chapter 2), provide diagnostic traits that allow us to distinguish the pubescent white oaks from the rest of the European white oaks, while there are not diagnostic traits useful in differentiating the various forms within Italian pubescent oaks. Thus, it is not possible to answers to the questions mentioned above in this thesis, because our data still not provide a clear response framework.

Again, however, I don't think that my doctoral thesis is providing a definitive response on the biosystematics issues (and consequently on the ecology and cenology) of the pubescent white oaks of southern Italy.

With the tools used for this study on the genetic, floristic and ecological field, I have concluded that at the actual state of knowledge; *'it is not possible to make a certain distinction between what are today reported as putative valid species within the group of the pubescent white oaks'*.

This does not mean that using a different approach or experimental protocols, currently unavailable, it will not be possible to arrive at a classification that could recognise unequivocally a level of distinct diversity that today is, unfortunately, only hypothesised.

Accordingly, I believe that it may be useful in the future to develop more specific genetic markers to investigate the European white oak group (within species rank). At the same time, I suppose that it will be crucial also a broader European sampling in the different geographic area and ecological niches, to obtain a more wide spectrum of data.

These two latter conditions I believe can concretely help to address lingering questions:

- How many species of pubescent oaks are there in Europe?
- How many phenomena of local speciation are not concluded yet?

Chapter 7. Paper contributions

During my experience in the PhD program I have contributed to collect and analyse data in other to taxonomic and vegetational research listed below:

CONSIDERAZIONI SU ALCUNI TAXA INCLUSI IN *PLANTAGO S*ECT. *MARITIMA* (*PLANTAGINACEAE*) IN ITALIA. PLANT BIOSYSTEMS.

- **Conte, A. L.,** Iamonico, D., Fortini, P., Valletta, A., & Di Pietro, R. (2017). Considerazioni su alcuni taxa inclusi in *Plantago S*ect. *Maritima* (*Plantaginaceae*) in Italia. Plant Biosystems, 137(1), 83-110.

PRELIMINARY CHARACTERIZATION OF THE *QUERCUS PUBESCENS* COMPLEX IN SOUTHERN ITALY USING MOLECULAR MARKERS. ACTA BOTANICA CROATICA

- Di Pietro, R., Di Marzio, P., Antonecchia, G., **Conte, A., L.,** Fortini, P (2020). Preliminary characterization of the *Quercus pubescens* complex in southern Italy using molecular markers. Acta Botanica Croatica, 78(2), 107-115. DOI: 10.2478/botcro-2019-0026.

THE DOMINANCE OF A COMPETITIVE GRASS MEDIATES THE EFFECTS OF SMALL-SCALE KARST TOPOGRAPHY ON PLANT SPECIES RICHNESS IN A MONTANE GRASSLAND.

Filibeck, G., Sperandii M., G., Bragazza L.,Bricca A., Chelli S., Maccherini S., Wellstein C., **Conte, A., L.,** Di Donatantonio M., Forte, T'ai G.W., Lazzaro L., Macchiavelli T., Maestri1, Roberta Marchesini, S. R., Marignani M., Midolo G., Oddi L., Rosati L., Silan G., Cancellieri L. (submitted, 2020). The dominance of a competitive grass mediates the effects of small-scale karst topography on plant species richness in a montane grassland.

Considerazioni su alcuni taxa inclusi in Plantago sect. *Maritima* (*Plantaginaceae*) in Italia[15]

Antonio Luca Conte[1], D. Iamonico[2], Paola Fortini[1], A. Valletta[3], Romeo Di Pietro[2]

[1]Department of Bioscience and Territory, University of Molise, 86090 Pesche (IS) Italy.
[2]Department PDTA, University of Rome Sapienza, 00196 Rome, Italy.
[3]Department of Environmental Biology, University of Rome Sapienza, 00196 Rome, Italy.

✉ Corresponding author, Romeo Di Pietro: romeo.dipietro@uniroma1.it

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[15] This paragraph reports a published article about the Sect. *Maritima*, Genus *Plantago* on Notiziario della Società Botanica Italiana, 1 (2): 165-198, 2017

Considerazioni su alcuni taxa inclusi in *Plantago* sect. *Maritima* (Plantaginaceae) in Italia

A.L. Conte, D. Iamonico, P. Fortini, A. Valletta, R. Di Pietro

La flora italiana include 28 entità di *Plantago* L. (Plantaginaceae) delle quali 3 endemiche (Conti et al. 2005). *Plantago* è un genere critico dal punto di vista tassonomico e la separazione dei diversi taxa su base morfologica è ancora una questione aperta, risultando difficoltosa l'identificazione delle varie specie in relazione alla riduzione e alla scarsità di caratteri diagnostici. Recentemente sono state chiarite alcune questioni nomenclaturali relative a *P. alpina* L., *P. strictissima* L., *P. subulata* L., *P. serraria* L, *P. serpentina* All. e *P. holosteum* Scop. (Di Pietro et al. 2013, Di Pietro, Iamonico 2014a, b, Iamonico et al. 2017).

A livello infragenerico sono riconosciuti 5 sottogeneri (Rønsted et al. 2002). Il subg. *Coronopus* include specie a distribuzione Mediterranea eccetto *P. maritima* L., presente anche in Europa centro-settentrionale, Asia, N- e S-America (Rahn 1996, Rønsted et al. 2002, Hassemer et al. 2017a). Il subg. *Coronopus* comprende due sezioni: sect. *Coronopus* e sect. *Maritima* H.Dietr. In relazione a problematiche tassonomiche ancora non risolte Il numero di specie riferibili alla sect. *Maritima* è ad oggi indefinibile, variando da 4 a 12 (vedi letteratura citata).

In base alle caratteristiche morfologiche, ecologiche e biogeografiche le specie di *Plantago* a foglie strette della sect. *Maritima* fanno capo a due diversi gruppi: 1) *alpina-maritima-serpentina*; 2) *subulata-humilis-holosteum*. Nell'ambito del gruppo *alpina- maritima-serpentina*, la recente tipificazione di *P. alpina* e *P. serpentina* ha consentito di avere alcuni punti di riferimento quantomeno nomenclaturali. In termini macro-morfologici ed ecologici le tre entità sono grossolanamente distinguibili come da tabella.

	Plantago alpina	***Plantago serpentina***	***Plantago maritima***
morfologia	Pianta alta 5-10(-15) cm. Foglie piane con evidente venatura centrale, 2-4 × 5-10(-15) cm, a lati paralleli e bruscamente covergenti all'apice. Guaine poco allargate alla base. Spighe 1-3 cm.	Pianta alta 5-15(-20) cm. Foglie lineari, rigide, 1-3 × 1-10(-20) cm., debolmente carnosette spesso con dentelli laterali. Guaina fogliare allargata alla base e brattee (acute) con evidenti ciglia sul margine. Spighe 2-5 cm.	Pianta alta (15-)20-40 cm. Foglie 0,3-1× 10-40 cm, evidentemente carnosette con possibili dentelli laterali. Guaina fogliare molto larga e brattee (arrotondate) con ciglia brevi sul margine. Spighe 2-8 cm.
ecologia	Fascia alpina e subalpina, praterie continue su suoli profondi (nardeti), vallette nivali a *Salix herbacea* e Festuco-Trifolieti.	Fascia montana, subalpina e alpina, praterie continue su suoli profondi sabbioso-argillosi, vallette nivali a *Trifolium thalii* e linee di impluvio sottoposte a fenomeni erosivi.	Fascia costiera, subcostiera e collinare su suoli a prevalente componente argillosa da subsalsi a debolmente alini. Aree calanchive nelle morfotipie colluviali.

Tuttavia la situazione in natura è più complessa e caratterizzata da ampie sovrapposizioni morfologiche e distributive tra le diverse entità, vedi ad esempio la dicotomia *P. alpina/P. serpentina* nell'alta quota dell'Appennino settentrionale e centrale (Alessandrini et al. 2003, Blasi et al. 2003), o quella *P. serpentina/P. holosteum/P. subulata/P. humilis* sui Monti Aurunci e nel Camposauro (Moraldo et al. 1990, Corazzi 2008).

Per quanto concerne il gruppo *P. subulata-P. humilis-P. holosteum* la situazione è addirittura più complessa in quanto si tratta di entità morfologicamente molto simili ma apparentemente ben separate a livello distributivo. Il gruppo di taxa ascrivibili a *P. subulata s.l.* è a gravitazione Mediterraneo centro-occidentale mentre *P. holosteum* è a gravitazione Mediterraneo centro-orientale. Sulla base di caratteri morfologici *P. subulata* s.l. si distingue da *P. holosteum* s.l. per le foglie più corte, la presenza di ciuffi di peli alla base delle stesse e la persistenza di diversi strati di foglie morte alla base dei fusti legnosi. Il recente studio di Hassemer et al. (2017b), propone la riduzione di *P. holosteum* e *P. grovesii* a sinonimi di *Plantago subulata*. Questa semplificazione tassonomico-nomenclaturale per entità isolate tra loro e aventi caratteristiche ecologiche differenti (scogliere calcaree per *P. grovesii*; pascoli montani della Calabria e della Sicilia, su substrati di vario tipo, per *P. humilis;* pascoli aridi collinari e montani su calcare dell'Appennino centrale, delle prealpi orientali e delle Dinaridi e pascoli aridi sui serpentini della Toscana per *P. holosteum*), nonché le sopramenzionate problematiche legate alla tassonomia del complesso *P. alpina-maritima-serpentina*, ci hanno indotto ad intraprendere uno studio a carattere morfometrico-anatomico-cariologico sui taxa appena menzionati. Lo studio ha riguardato campioni raccolti esclusivamente in territorio italiano. Tali campioni (attualmente conservati in HFLA) derivano da raccolte effettuate dagli autori negli ultimi 15 anni. Per questa prima indagine sono state considerate le seguenti località: *P. alpina*: Alpi occidentali (Colle dell'Agnello); *P. serpentina*: Alpi occidentali (Val Varaita); App. settentrionale (Alpe Tre Potenze); App. centrale (Monti della Laga, Sirente-Velino, Gran Sasso); App. meridionale (Monti di Orsomarso); *P. holosteum*: App. ligure (Val D'Aveto); Toscana (Monterufoli, Colline Pisane); App. centrale (Monti Aurunci, Monti Sibillini); App. meridionale (Monti della Maddalena); *P. subulata* (Isole Tremiti); *P. humilis* (Calabria: Sila; Sicilia: Madonie); *P. grovesii* (Torre dell'Orso-Otranto). Sono stati esaminati 38 caratteri (27 qualitativi, 11 quantitativi) e la loro variabilità rappresentata tramite *clustering*, PCA, Scatter-Plot e Box-Plot. Le osservazioni da noi effettuate, da ritenere preliminari, mostrano foglie di lunghezza superiore in *P. holosteum* rispetto a *P. subulata*, *P. humilis* e *P. grovesii*. Quest'ultima, rispetto a tutte le altre popolazioni italiche, mostra una evidente presenza di ciuffi di peli biancastri alla base delle foglie della rosetta e lacinie corolline mediamente più piccole. Le popolazioni di *P. alpina* delle Alpi occidentali differiscono in maniera netta e evidente da quelle di *P. serpentina* raccolte nelle stesse aree, mentre le popolazioni delle alte quote Appenniniche (sia settentrionale che centrale) mostrano tutte maggiore affinità verso *P. serpentina*. Interessante è la presenza sui Monti della Laga di popolazioni di *P. serpentina* con foglie abbondantemente pelose anche sulla lamina. L'analisi cariologica ha individuato popolazioni di *Plantago humilis* (Sila) aventi numero cromosomico $2n = 12$ (diploidi) rispetto al corredo tetraploide $2n = 24$ conosciuto ad oggi (Brullo et al. 1985, Aquaro et al. 2004). Tale dato smentirebbe la teoria che il corredo tetraploide di *P. humilis* rispetto a quello diploide di *P. subulata* fosse legato a fattori altitudinali. Un altro risultato di interesse è la presenza di un triplo numero cromosomico per *P. serpentina*: $2n = 12$ Monti della Laga piano montano, $2n = 24$ Monti della Laga piano subalpino-alpino e $2n = 14$ Parco Nazionale del Pollino-Orsomarso.

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AUTORI

Duilio Iamonico, Romeo Di Pietro (romeo.dipietro@uniroma1.it), Dipartimento PDTA, Sapienza Università di Roma, Via Flaminia 72, 00196 Roma
Antonio Luca Conte, Paola Fortini, Dipartimento di Bioscienze e Territorio, Contrada Fonte Lappone, Pesche (Isernia)
Alessio Valletta, Dipartimento di Biologia Ambientale, Sapienza Università di Roma, Piazzale Aldo Moro 5, 00185 Roma
Autore di riferimento: Romeo Di Pietro

Preliminary characterisation of the *Quercus pubescens* complex in southern Italy using molecular markers[16]

Romeo Di Pietro[1], Piera Di Marzio[2], Gaby Antonecchia[2], Antonio Luca Conte[2], Paola Fortini[2]

[1]Department PDTA, University of Rome Sapienza, 00196 Rome, Italy.
[2]Department of Bioscience and Territory, University of Molise, 86090 Pesche (IS) Italy.

✉ Corresponding author, Paola Fortini: fortini@unimol.it

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Preliminary characterization of the *Quercus pubescens* complex in southern Italy using molecular markers

Romeo Di Pietro[1], Piera Di Marzio[2], Gaby Antonecchia[3], Antonio Luca Conte[2], Paola Fortini[2*]

[1] Department of Planning, Design, Architecture technology (PDTA), Herbarium Flaminio, Sapienza University of Rome, Rome, Italy

[2] Department of Bioscience and Territory, University of Molise, Pesche (IS), Italy

[3] Ministry of Education, University and Research (MIUR), Rome, Italy

Abstract – *Quercus pubescens* s.l. is a group of taxonomically intricate and highly debated deciduous white oaks widely distributed in southern Europe. The Apulia region occupies the south-easternmost part of the Italian Peninsula; the land-use pattern is based on extensive agricultural systems and only 10% is covered by forests that are mainly composed of oak woods. It is the region in Italy showing the highest number of oak species, among which four putative species of the *Quercus pubescens* group, have been reported in floras and checklists with uncertain taxonomic value because of the overlapping of diagnostic characters. In this paper, we carried out a molecular analysis on natural populations of *Q. pubescens* s.l. distributed throughout the Apulia region. Individuals from 24 pubescent oak populations were sampled and each tree was genotyped at 11 polymorphic microsatellite markers. Overall, the average expected heterozygosity (H_e) was 0.629, and the allelic richness (A_r) ranged between 2.130 and 7.187. No differentiation was observed among the populations investigated, and the genetic differentiation coefficient (F_{ST}) was 0.036. Gene flow among populations was found to be relatively high (Nm = 6.664). From a taxonomic point of view, the possibility of the coexistence of more than one species among the Apulian pubescent oaks reported in the taxonomic and syntaxonomic literature is not supported by the results of this molecular analysis.

Key words: genetic diversity; Italy; population structure; *Quercus pubescens*; SSR; taxonomy

Introduction

The genus *Quercus* L. subgenus *Quercus* has a wide distribution in the Northern Hemisphere, especially in central and southern Europe, where *Quercus* species form important forest communities in the Temperate and the Mediterranean bioclimatic regions (Nixon 1993, Govaerts and Frodin 1998).

Italy is the country showing the highest oak species diversity in Europe, although there is still incomplete agreement on the exact number of oak taxa occurring in the territory. Most of the taxonomic uncertainty regarding the Italian (and European) oaks concerns the so-called white oaks and in particular the pubescent oaks (*Quercus pubescens* s.l. subgen. *Quercus* Sect. *Quercus*). The most recent Italian flora and checklists (Pignatti et al. 2017, Bartolucci et al. 2018) report various pubescent oaks in southern Italy (*Q. amplifolia* Guss., *Q. apennina* Lam., *Q. congesta* C. Presl, *Q. dalechampii* Ten., *Q. leptobalana* Guss., *Q. ichnusae* Mossa, Bacch. et Brullo, *Q. humilis* Mill., *Q. virgiliana* Ten.) which are all considered Steno-Mediterranean vicariant species of *Q. pubescens* Willd.

Apulia is the easternmost Italian administrative region and all Italian oak species, both evergreen and deciduous, occur in this region with the exception of *Q. petraea* (Matt.) Liebl. Despite this richness in oak species and a wide potential range for the oak woodlands, only 10% of the Apulian territory is covered by forests. The *Q. pubescens* s.l. forests are the most widespread and can be found in the flooded depressions of the plain, in the limestone plateaus and on the sub-montane rocky slopes.

Molecular marker studies have not yet been published for the genus *Quercus* in the Apulian region. However, the

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* Corresponding author e-mail: fortini@unimol.it

evaluation of genetic variation can be of great importance for the conservation and management of forest ecosystems, especially in areas particularly vulnerable to climatic change (Peñuelas et al. 2017) and in forests whose original extent has been significantly reduced. This is the case of the oak forests of the Apulia region. In fact, the dry Mediterranean climate that characterizes this region places it at risk of desertification if the climate should experience a further increase in aridity. Moreover, the widespread practice of extensive agriculture (wheat, olive and vine) during the last century led to the destruction of most of the natural forest resources (Biondi et al. 2010).

Several reports on the population genetics of the white oaks *Q. robur* L., *Q. petraea*, *Q. pubescens* have been published for south-eastern Europe in the last two decades (Franjić et al. 2006, Curtu et al. 2007a, Jerše and Batič 2007, Slade et al. 2008, Curtu et al. 2009, Ballian et al. 2010, Enescu et al. 2013, Gailing et al. 2013). In Italy, only few data are available. Fineschi et al. (2002) and Fineschi and Vendramin (2004) presented a study on the genetic diversity of the Italian white oaks analyzing chloroplast DNA. Subsequently, several studies that compared leaf morphology and molecular data on some white oaks populations of Central Italy have been published (Fortini et al. 2009, 2013, 2015).

In the most recent papers published on the Apulia forests (Biondi et al. 2004, Di Pietro and Misano 2009) the following pubescent oaks were reported in the phytosociological tables: *Q. pubescens*, *Q. virgiliana*, *Q. amplifolia* and *Q. dalechampii*. A recent study (Di Pietro et al. 2016) statistically analyzed 25 morphological characters of leaves and fruits within 24 pubescent oak populations in the Apulia region, in order to identify possible diagnostic traits useful for taxonomic differentiation. The results provided no evidence for differentiation among tree individuals based on comparisons of morphological traits. In the present study plant material from the same pubescent oak populations was used to analyze their genetic diversity, structure and gene flow. The aims of this study are twofold. First, to assess whether any group of genetic structure could be identified among oak individuals and/or populations, notwithstanding the morphological and taxonomical uniformity highlighted in the previous morphological work (Di Pietro et al. 2016). Second, to investigate the pattern of genetic diversity in the Apulian pubescent oaks in order to provide useful information for further studies.

Material and methods

Study area

The Apulia region is located in the south-eastern part of the Italian Peninsula where it is largely open to the Adriatic and Ionian Seas. It is slightly sloping, with more than 60% of the territory occurring below 200 m a.s.l. Five physiographic units can be distinguished in the Apulia region and these are the Daunian sub-Apennine (1150 m), the Gargano promontory (1080 m), the Murgian Plateau (680 m), the Salento Peninsula and the large plain Tavoliere delle Puglie. The bedrock of the mountainous systems is mainly composed of Cretaceous limestone and Paleocenic calcarenites. Marls occur in the lower parts of the Daunian sub-Apennine and in the Tavoliere plain together with conglomerates and Pleistocenic and Holocenic sand deposits. The bioclimate is Thermo-Mediterranean and Meso-Mediterranean with the Temperate region occurring only in the sub-and lower-montane belts. The rainiest areas are the Gargano, the Daunian sub-Apennine and the south-eastern part of the Salento Peninsula (slightly over 800 mm year^{-1}). Annual precipitation values on average of less than 500 mm year^{-1} are recorded in the western side to the Murgian Plateau and in the Tavoliere plateau whereas the remaining portion of the territory exhibits an average annual rainfall of between 500 and 700 mm year^{-1} (Blasi and Michetti 2007, Cotecchia et al. 2014). As far as the climax vegetation is concerned almost the entire Apulian territory would potentially be covered by oak forests except for some scattered coastal and subcoastal areas dynamically linked to the *Pinus halepensis* Mill. woods and maquis, and the inner part of the Gargano promontory where the wide "Foresta umbra" beech wood occurs (Biondi et al. 2004, 2010).

Population sample information

Individual mature oak trees were randomly selected in each population trying to maintain a distance of at least 20 meters from each other. Leaves were randomly collected from 379 individuals within 24 populations (subdivided in squares 50 × 50 m), which covered approximately uniformly the physiographic units of the Apulia region (Fig. 1, Tab. 1, On-line Suppl. Tab. 1). Both the individuals and the populations they belong to are the same on which the morphological analysis of leaves and acorns was performed in the paper by Di Pietro et al. (2016). The collected oak specimens were dried on silica gel, and stored at room temperature until analysis. Voucher specimens for each individual sampled were stored at the Herbarium of the University of Molise.

DNA extraction, EST-SSR amplification

Total genomic DNA was extracted from 0.5 g of dried leaves from all samples using spin columns of "Invisorb® Spin Plant Mini Kit" and following the protocol of the manufacturer.

Eleven microsatellite loci or simple sequence repeats (EST-SSRs), developed by Durand et al. (2010) (PIE020, PIE102, PIE152, PIE215, PIE223, PIE227, PIE239, PIE242, PIE243, PIE267, PIE271) and polymorphic in white oaks (Guichoux et al. 2011, Antonecchia et al. 2015) were selected. Polymerase chain reaction (PCR) amplification was performed in a single multiplex reaction in a DNA Engine Tetrad (MJ Research Bio-Rad) thermocycler and PCR products were run on an ABI 3730xl capillary sequencer (Applied Biosystems) using Genescan 600 LIZ internal size standard (Applied Biosystems). Alleles were scored using STR and software version 2.3.106 (Toonen and Hughes 2001) and al-

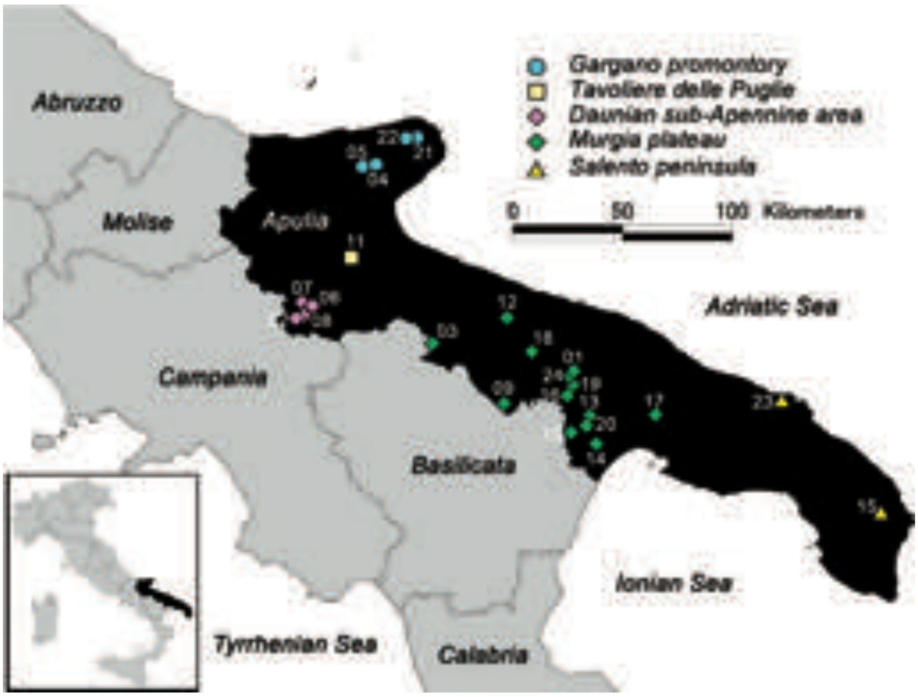

Fig. 1. Locations of the 24 oak collection stands.

Tab. 1. *Quercus* species composition of the 24 oak populations (according to Biondi et al. 2004, Biondi and Guerra 2008, Di Pietro and Misano 2009) with number of genotyped individuals and coordinates.

Population code	Analyzed individuals	Genotyped individuals	Latitude (N)	Longitude (E)	*Quercus* species
Pop01	16	16	4524805	650840	*Q. pubescens*
Pop02	16	16	4557220	526723	*Q. pubescens*
Pop03	16	16	4624669	567854	*Q. pubescens*
Pop04	16	16	4624043	561581	*Q. virgiliana*, *Q. dalechampii*, *Q. pubescens*
Pop05	16	15	4562416	534533	*Q. virgiliana*, *Q. dalechampii*, *Q. pubescens*
Pop06	16	15	4564231	529921	*Q. pubescens*
Pop07	16	16	4558496	530175	*Q. pubescens*
Pop08	16	16	4512469	618357	*Q. pubescens*
Pop09	18	16	4497035	647716	*Q. pubescens*
Pop10	16	16	4582747	553553	*Q. pubescens*, *Q. dalechampii*, *Q. virgiliana*
Pop11	16	16	4551101	622237	*Q. virgiliana*, *Q. dalechampii*, *Q. amplifolia*
Pop12	15	15	4504016	656599	*Q. pubescens*
Pop13	12	12	4491270	658881	*Q. pubescens*, *Q. dalechampii*, *Q. virgiliana*
Pop14	16	16	4446957	278106	*Q. virgiliana*, *Q. amplifolia*, *Q. dalechampii*
Pop15	15	15	4514201	647317	*Q. pubescens*
Pop16	16	11	4502657	686693	*Q. pubescens*
Pop17	16	13	4534971	632507	*Q. pubescens*, *Q. dalechampii*, *Q. virgiliana*
Pop18	16	14	4523011	649110	*Q. dalechampii*, *Q. virgiliana*, *Q. pubescens*
Pop19	16	14	4499728	654902	*Q. pubescens*
Pop20	17	14	4635503	582284	*Q. pubescens*, *Q. dalechampii*, *Q. virgiliana*
Pop21	16	16	4504890	744270	*Q. virgiliana*, *Q. dalechampii*, *Q. pubescens*
Pop22	14	11	4518821	650238	*Q. virgiliana*, *Q. dalechampii*, *Q. pubescens*
Pop23	16	6	4540867	597285	*Q. pubescens*
Pop24	16	7	4635677	587298	*Q. pubescens*

lele binning was performed as described in Guichoux et al. (2011). MICROCHECKER (Van Oosterhout et al. 2004) was used to check for the presence of null alleles and to check for potential problems related to allele dropout with 1000 randomizations and a 95% confidence interval.

Data analysis

Polymorphic EST-SSR markers were used to analyze the genetic diversity of 338 *Q. pubescens* s.l. individuals in 24 populations. EST-SSR amplification was unsuccessful for 41 of the 379 individuals sampled. Two populations (Pop23 and Pop24) were not included in the statistical analyses because of the low number of amplified individuals.

The basic statistics for each locus: number of alleles (NA), number of individuals (N), observed heterozygosity (H_o), expected heterozygosity (H_e), and number of private alleles (PA) were computed using the statistical program GenAlEx version 6.503 (Peakall and Smouse 2012).

Polymorphic information content (PIC), deviation from Hardy-Weinberg equilibrium (HW), and null allele frequency (F(Null)) were computed for each locus using Cervus software version 3.0.7 (Kalinowski et al. 2007).

The basic statistics for each population: number of different alleles, number of effective alleles, allelic richness, observed heterozygosity, expected heterozygosity, index of fixation (F_{IS}) calculated as F = 1 – (Ho / He) and number of private alleles, were computed using Arlequin software version 3.5.2.2 (Excoffier and Lischer 2010). Allelic richness per locus and population were computed using FSTAT version 2.9.4 (Goudet 2003).

Analysis of molecular variance (AMOVA) was performed using GenAlEx in order to partition the total microsatellite diversity, among and within populations, and within the individuals of each *Q. pubescens* s.l. population, based on 11 EST-SSR markers loci. The variance components were statistically tested by non-parametric randomization tests of significance based on 1000 bootstraps. Gene flow among populations was estimated using the indirect method based on the number of migrants per generation (Nm) using the formula, $Nm = 0.25\ (1 - F_{ST}) / F_{ST}$ (Slatkin and Barton 1989). To investigate population differentiations, pairwise F_{ST} between all pairs of populations was computed, F_{ST} was calculated according to Weir and Cockerham (1984) in program FSTAT 2.9.4.

Principal coordinate analysis (PCoA) with data standardization based on Nei's genetic distance was performed using GenAlEx.

Bayesian clustering analysis, using STRUCTURE software version 2.3.4 (Pritchard et al. 2000, 2010)**,** with population IDs as Sampling Location Information, has been carried out. We opted for the admixture with correlated allele frequencies model between populations. We used a burn-in period of 25,000 and a number of MCMC of 100,000 cycles. Ten runs for each K (K = number of possible clusters) value were done to test the consistency of the results. Each (K) value was tested from K = 1 to K = 10. The optimum K value was predicted using the web-based software STRUCTURE HARVESTER web version 0.6.94 which implements the Evanno method (Earl and vonHoldt 2012). Individuals with probabilities above 0.80 ($Q \geq 0.800$) were assigned as putative purebred while all the other individuals were assigned as "putative hybrids" although the term "hybrid" could be a little misleading since no plant material of possible parental species was collected. For this reason we have opted for using the much more neutral term "off-type" in the rest of the text.

BOTTLENECK software version 1.2 (Cornuet and Luikart 1996) was used to detect the likelihood of a bottleneck effect. Heterozygosity excesses were displayed, identified based on the estimates of multilocus genotypes calculated by using the Wilcoxon signed rank tests and evaluating departures from the ratio 1:1 deficiency/excess (Cornuet and Luikart 1996, Luikart and Cornuet 1998, Piry et al. 1999). A two-phase mutation model (TPM) and a stepwise mutation model (SMM) for Wilcoxon signed-rank tests were used considering a 90% SMM proportion and TPM with a variance of 10, and 1000 repeats.

Results

SSR polymorphism and genetic diversity

All the loci were polymorphic ($Pj = q \leq 0.99$). The total number of alleles identified was 100 out for the 325 individuals analyzed. The allele fragment sizes (Tab. 2) matched with the relative SSR reference size, as described in Guichoux et al. (2011). The number of alleles per locus (k) ranged between 4 and 17, with an average of 11 alleles. The PIC values varied from 0.233 (PIE227) to 0.879 (PIE152). According to (Hildebrand et al. 1992) eight EST-SSR loci were found to be informative (PIC ≥ 0.5), whereas two were found to be slightly informative (PIC < 0.4). The observed heterozygos-

Tab. 2. Allelic diversity of eleven microsatellite loci scored in 325 individuals of *Quercus pubescens* s.l.; S – size range fragments (bp); NA – Number of alleles at the locus, Ar – Allelic richness per locus, N – Number of individuals typed at the locus, H_o – Observed heterozygosity, H_e – Expected heterozygosity, PIC – polymorphic information content, HW – Significance of deviation from Hardy-Weinberg equilibrium (Hardy-Weinberg equilibrium test chi-square value, p value and significance with Bonferroni correction: ns = not significant, * = P < 0.05; ** = P < 0.01; *** = P < 0.001), F(Null) – Null allele frequency estimate, F_{IS} – Fixation index (* = P < 0.05).

Locus	S	NA	Ar	N	H_o	H_e	PIC	HW	F(Null)	F_{IS}
PIE020	97–109	7	3.386	312	0.583	0.585	0.537	35.434***	–0.026	–0.067
PIE102	139–167	13	5.367	321	0.523	0.750	0.726	87.243 ***	0.187	0.279*
PIE152	228–260	17	7.187	314	0.834	0.890	0.879	3.756 ^{ns}	0.032	0.043*
PIE215	185–218	12	6.295	281	0.384	0.707	0.673	193.668***	0.311	0.417*
PIE223	197–234	11	5.980	318	0.811	0.822	0.803	0.525 ^{ns}	0.006	–0.002
PIE227	156–165	4	3.387	323	0.223	0.251	0.233	3.776 ^{ns}	0.060	0.095*
PIE239	69–93	9	4.689	302	0.248	0.435	0.420	56.089***	0.274	0.399*
PIE242	102–132	14	5.924	318	0.748	0.841	0.821	16.677^{ns}	0.058	0.093*
PIE243	204–224	10	4.392	282	0.688	0.683	0.649	37.002***	–0.029	–0.038
PIE267	84–106	12	4.029	313	0.425	0.667	0.622	82.591***	0.227	0.334*
PIE271	182–204	12	2.130	311	0.949	0.843	0.823	37.040***	–0.063	–0.153
Mean	–	11	–	308.64	0.583	0.679	0.653	–	0.094	–
Standard error	–	1.053	–	4.386	0.074	0.058	0.059	–	0.040	–

ity (H_o) for microsatellite loci ranged from 0.223 (PIE227) to 0.949 (PIE271), and the average was 0.583. A high level of H_o was detected in seven EST-SSR loci ranging from 0.523 to 0.949, whereas four loci detected low H_o with values ranging from 0.223 to 0.425. The expected heterozygosity (H_e) ranged from 0.251 to 0.890 with an average value of 0.679 and was higher than H_o for all the microsatellite loci, except for PIE243 and PIE271. The Hardy-Weinberg exact test for all populations (HW) revealed that seven loci (PIE020, PIE102, PIE 215, PIE239, PIE243, PIE267, PIE271) exhibited significant deviation from Hardy–Weinberg equilibrium ($P<0.001$) whereas four loci (PIE152, PIE223, PIE227, PIE242) did not show significant departures from Hardy-Weinberg equilibrium.

The indexes of genetic diversity of the 22 *Q. pubescens* s.l. populations are summarized in Tab. 3. The number of different observed alleles (Na) and effective alleles (Ne) averaged across all loci ranged from 4.727 (Pop22) to 7.182 (Pop09) and 2.559 (Pop22) to 4.363 (Pop20), respectively. Nine private alleles (PA) was found and these are distributed within ten individuals in turn distributed in eight oak populations (two other private alleles were found in Pop24; On-line Suppl. Tab. 2). The allelic richness (Ar), ranged between 3.865 (Pop3) and 5.171 (Pop20). The average observed heterozygosity (aH_o) ranged from 0.487 (Pop03) to 0.665 (Pop11) with mean value of 0.583. The average expected heterozygosity (aH_e) ranged from 0.510 (Pop03) to 0.709 (Pop09) with mean value of 0.629. Overall, the observed heterozygosity showed mostly slightly lower values than the expected heterozygosity. The mean fixation index (F_{IS}) values are close to zero for all populations (ranging from 0.058 in Pop11 to 0.188 in Pop21) that are the values expected under a random mating.

Tab. 3. Genetic diversity parameters for the *Quercus pubescens* s.l. populations (Pop) analyzed through eleven microsatellite loci. n – number of individuals; N_a – average number of different alleles; N_e – average number of effective alleles; Ar – allelic richness; aH_e – average expected heterozygosity; aH_o – average observed heterozygosity; F_{IS} – average of fixation index tested by 1023 permutation of gene copies between individuals within population (* = $P < 0.05$); PA – number of private alleles.

Pop	n	N_a	N_e	Ar	aH_o	aH_e	F_{IS}	PA
Pop01	16	6.182	3.641	4.642	0.581	0.604	0.035	0
Pop02	16	5.091	3.353	4.064	0.498	0.584	0.125*	0
Pop03	16	4.818	2.968	3.865	0.487	0.510	0.029	0
Pop04	16	6.364	3.684	4.605	0.572	0.638	0.094*	0
Pop05	15	5.182	3.097	4.192	0.604	0.610	-0.036	1
Pop06	15	5.182	3.055	3.940	0.529	0.590	0.037	0
Pop07	16	5.636	3.213	4.156	0.557	0.544	-0.059	0
Pop08	16	6.455	3.888	4.775	0.577	0.632	0.086*	0
Pop09	16	7.182	4.166	4.903	0.605	0.709	0.155*	0
Pop10	16	6.455	3.979	4.601	0.587	0.656	0.093*	0
Pop11	16	6.000	3.369	4.313	0.665	0.614	-0.112	0
Pop12	15	5.909	3.790	4.420	0.628	0.655	0.025	1
Pop13	12	5.545	3.883	4.760	0.595	0.705	0.092	2
Pop14	16	6.091	3.749	4.487	0.655	0.648	-0.018	1
Pop15	15	5.727	3.503	4.583	0.596	0.634	0.066	0
Pop16	11	5.273	3.400	4.606	0.627	0.647	0.006	1
Pop17	13	6.091	3.700	4.560	0.580	0.639	0.062	0
Pop18	14	5.909	3.688	4.651	0.605	0.642	0.005	1
Pop19	14	6.545	3.955	4.841	0.583	0.669	0.064	0
Pop20	14	7.091	4.363	5.171	0.617	0.692	0.120*	1
Pop21	16	6.000	3.753	4.510	0.557	0.684	0.116*	1
Pop22	11	4.727	2.559	3.941	0.527	0.540	-0.129	0
Mean	14.8	5.884	3.580	4.481	0.583	0.629	0.039	

Population genetic structure and gene flow

The overall population differentiation degree is reported in Tab. 4. AMOVA analysis showed that 3.6% ($P < 0.001$) of the genetic variations was among populations while 96.4% was within populations. The majority of molecular variance was partitioned within individuals (78.5%, $P < 0.01$) while 17.9% ($P < 0.01$) of variance was found to occur among individuals. The pairwise F_{ST} matrix between populations is shown in On-line Suppl. Tab. 3; eleven population pairs exhibited negative values but all of them showed no significant p values (all largely > 0.05). The differentiation coefficient between population pairs having significant P values showed that Pop11 (Incoronata-Tavoliere) and Pop15 (Palmariggi-Salento) was the smallest ($F_{ST} = 0.0025$; $P \leq 0.001$); the differentiation coefficient between Pop02 (Deliceto-Daunian sub-Apennine) and Pop22 (Vico del Gargano-East Gargano) was the largest ($F_{ST} = 0.1053$; $P < 0.01$).

Nei's genetic distance among the populations belonging to the five units are graphically illustrated in Fig. 2, constructed on the basis of principal coordinate analysis (PCoA with the physiographic units superimposed). The first two axes of the PCoA explained 42.26% of the variation. The scattergram did not show an evident correlation between the physiographic units and the genetic similarities of the populations. In fact, populations belonging to four different physiographic units are closely associated along the first axis in the left part of the diagram. The Mantel test comparing the matrices of geographic and genetic distances among populations did not evidence any correlation between these two variables.

Tab. 4. Analysis of molecular variance among populations, within populations, and within individuals of *Quercus pubescens* s.l. populations; Df – degree of freedom, SS – sum of squares, MS – mean squares, P – probability based on standard permutation across the full data set, Nm –average estimate of gene flow among populations.

Source of variation	Df	SS	MS	Estimated variation	Percentage variation %	F-statistics	P-value	Gene flow
Among Populations	21	180.496	8.595	0.141	3.6	Fst = 0.036		Nm = 6.664
Among Individuals	303	1346.241	4.443	0.695	17.9	Fis = 0.185	0.001	
Within Individuals	325	992.500	3.054	3.054	78.5	Fit = 0.215		
Total	649	2519.237		3.889	100			

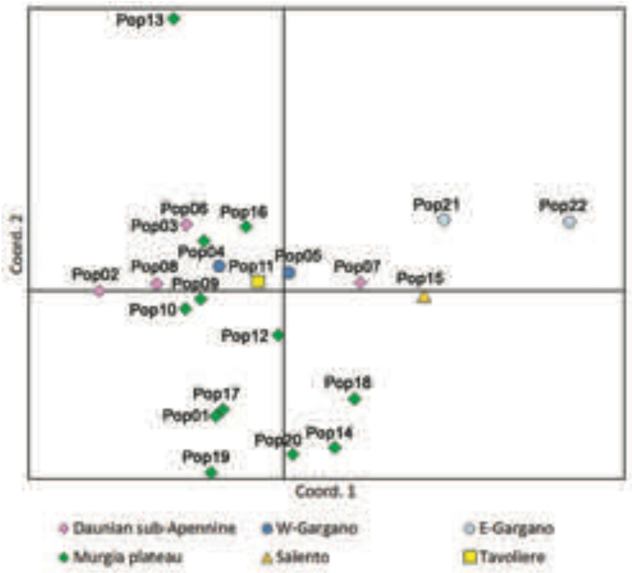

Fig. 2. Principal Coordinate Analysis (PCoA) via Distance matrix (Nei Genetic Distance) with data standardization. Daunian sub-Apennine populations: Pop02, 06, 07, 08; W-Gargano populations: 04, 05; E-Gargano populations: 21, 22; Murgia plateau populations: 01, 03, 09, 10, 12, 13, 14, 16, 17, 18, 19, 20; Salento population: 15; Tavoliere population: 11.

When STRUCTURE was run using population IDs as sampling location information, a maximum value of the rate of change in the log probability of data was revealed at K = 2, using Evanno's method (Evanno et al. 2005) (On-line Suppl. Fig. 1).

The individual membership proportion determined by Bayesian clustering analysis suggested a subdivision of the whole data-set of oak individuals (325) into two genetic clusters: a first cluster including 305 pure individuals (94.15%) and a second one composed of 19 off-type individuals (5.85%) (Fig. 3a). The distribution of the 19 off-types involved 13 populations out of the 22 investigated (Fig. 3b, On-line Suppl. Tab. 4). Population 13, where the sampled individuals were selected from a mixed wood with *Q. pubescens* and *Q. robur* that developed in a humid area, is the population showing the highest number (4) of off-types. Populations 2, 9, and 10 count two off-types each and relate to *Q. pubescens* s.l. woods with the occurrence of *Q. frainetto*.

The Wilcoxon sign-rank test showed no significant results as regards bottleneck effect in all the populations analyzed, using both TPM and SMM models. The observed number of loci with heterozygosity excess (obs LH_{exc}) was found to be always lower than the expected number of loci with heterozygosity excess (exp LH_{exc}) except for population 13 where "obs LH_{exc}" is about 1.5 times greater than "exp LH_{exc}". However, the Wilcoxon sign-rank test was not significant (Tab. 5).

Discussion

The data-set exhibited the following levels of genetic diversity at the microsatellite loci examined: mean expected heterozygosity (H_e) = 0.679, mean observed heterozygosity (H_o) = 0.583, average number of alleles per locus (K) = 11.0 (from 4 to 17 alleles). Four (PIE102, PIE215, PIE239, PIE267), out of the eleven loci investigated, showed a positive F_{IS} (0.279-0417) which turned out to be statistically significant (Tab. 2). It is known that the fixation of alleles could be favored by directional selection (Andolfatto 2001), or be the results of genetic drift. Molecular studies regarding oth-

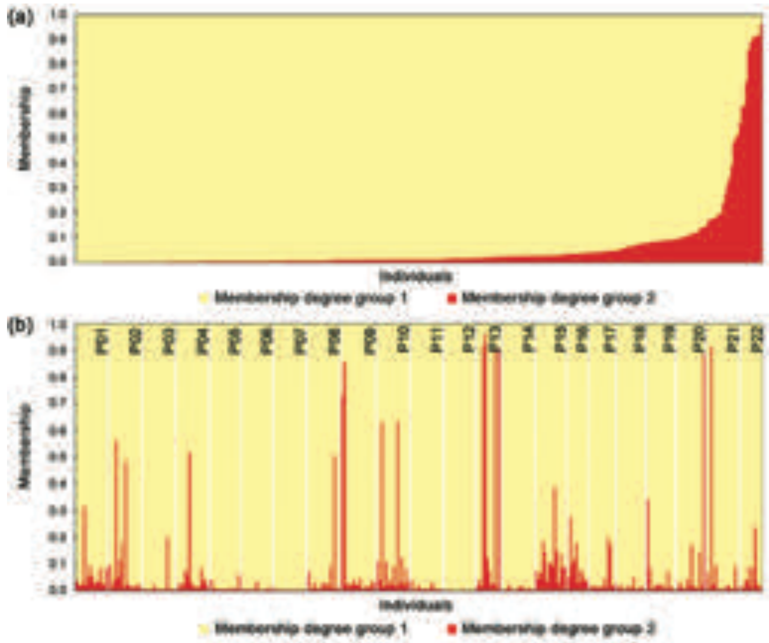

Fig. 3. Genetic assignment obtained with STRUCTURE clustering analysis. Oak individuals are distributed in progressive order along the horizontal axis while the vertical axis expresses the group membership percentage degree per single individual per K=2. Clustering of all the data-set oak individuals into two different genetic clusters with decreasing membership degrees for group 2 and increasing degrees for group 1 (a). Clustering of all the data-set oak individuals ordered per populations; the latter separated by white lines (b).

Tab. 5. Results of tests for genetic bottlenecks using the two-phase mutation model (TPM) and a stepwise mutation model (SMM), and the Wilcoxon sign-rank tests for heterozygosity excess; the number of polymorphic loci for all populations (Pop) is 11; obs LH_{exc} – observed number of loci with heterozygosity excess; exp LH_{exc} – expected number of loci with heterozygosity excess; P – probability of no significant heterozygosity excess.

	TPM			SMM		
Pop	obs LH_{exc}	exp LH_{exc}	P	obs LH_{exc}	exp LH_{exc}	P
Pop01	4	6.46	0.9492	2	6.31	0.9954
Pop02	6	6.44	0.7114	4	6.43	0.8398
Pop03	4	6.43	0.9385	4	6.43	0.9585
Pop04	4	6.37	0.9126	4	6.37	0.9585
Pop05	3	6.29	0.8608	3	6.29	0.9263
Pop06	5	6.39	0.7676	4	6.35	0.8799
Pop07	4	6.36	0.9585	4	6.38	0.9731
Pop08	5	6.49	0.9492	4	6.58	0.9663
Pop09	3	6.55	0.9492	2	6.50	0.9976
Pop10	5	6.27	0.7676	3	6.36	0.8970
Pop11	2	6.39	0.9895	2	6.49	0.9966
Pop12	5	6.49	0.5845	5	6.57	0.7114
Pop13	8	6.52	0.1602	8	6.61	0.2598
Pop14	4	6.59	0.8608	4	6.58	0.9585
Pop15	6	6.58	0.7676	5	6.51	0.8608
Pop16	4	6.59	0.9263	4	6.55	0.9663
Pop17	5	6.46	0.8799	2	6.51	0.9919
Pop18	3	6.47	0.9126	3	6.50	0.9126
Pop19	4	6.39	0.9126	4	6.50	0.9492
Pop20	4	6.48	0.8799	3	6.59	0.9919
Pop21	6	6.56	0.6812	4	6.57	0.8970
Pop22	3	6.56	0.9663	2	6.53	0.9954

er oak species stated that the genetic variation removed by genetic drift would affect the genome rather uniformly (Alberto et al. 2010). This would not seem to be the case of the Apulian pubescent oak populations, since the other EST-SSR loci examined showed only slightly positive values of F_{IS}, or even negative ones. Due to the relatively low number of sampled individuals per population, it cannot be completely excluded that these results could be addressed, at least in part, to the sampling effect. However, there is no uniformity of views at present on the minimum number of individuals per population that should be sampled to detect "reliable" results for genetic differentiation. Hale et al. (2012) stated that the number of individuals per population should be established using a "case by case" approach. On the other hand the genetic literature on this issue shows a wide range of opinions and experiences from which it emerges that the number of individuals sampled can be highly variable. In many cases it was considered as acceptable to take a minimum of 10 to 30 individuals. For some *Pinus sylvestris* L. populations in the Baltic area, the number of 20-25 individuals was found to be large enough to detect all the alleles (Danusevičius et al. 2016) whereas other studies have even considered significantly lower numbers (Kitamura et al. 2017, Rinaldi et al. 2019). In contrast, in Kalinowski (2005) it was shown particularly clearly that when F_{ST} is less than 0.01 it can be useful to sample up to 100 individuals. As far as we are concerned, we believe that these results, although deriving from the analysis of populations composed of a seemingly limited number of samples, can still provide interesting information that should not be completely ignored.

The highest allelic variability was found in locus PIE152 with 17 alleles and PIC = 0.879. A similar variability was found in other pubescent oak populations investigated in southern Italy and central Italy (Antonecchia 2015) where locus PIE152 exhibited the highest polymorphic degree and in particular the highest values of allelic richness for *Q. pubescens*. The still insufficient knowledge about the *Q. pubescens* genome do not allow it to be established if the high variability in locus PIE152 might be associated with adaptive traits. More in-depth investigations would be needed to clarify this point.

The level of genetic variability within the pubescent oak populations of the Apulia region was here found to be lower than that reported for *Q. pubescens* in other countries. Curtu et al. (2007b) reported $aH_e = 0.891$, Ar = 17.8 on a study based on 6 microsatellite loci for 73 *Q. pubescens* individuals while Enescu et al. (2013) reported aH_e=0.847, k = 22 and Ar = 14.22 in a 7 microsatellite loci study in a geographically related area. The heterozygosity values reported in these studies, however, would not be directly comparable with those obtained by us due to the use of different type of markers (genomic SSRs vs. EST-SSRs). Some authors (Durand et al. 2010, Parthiban et al. 2018) showed that the use of EST-SSRs highlights the values of polymorphism slightly lower than those obtained from the use of genomic SSRs. However, there are some studies on the *Quercus* genus in which both these types of markers were tested. Curtu et al. (2011) investigated the genetic variability of 65 individuals of *Q. pubescens* using 7 microsatellite loci, of which 5 gSSR and 2 EST-SSRs. The overall average values for the genetic parameters in issue were the following: $aH_e = 0.859$, k = 17, Ar = 16.5. Calculating the values of these parameters solely for the two EST-SSRs markers we obtained values ($aH_e = 0.819$, k = 17, Ar = 16.8) that are lower than those obtained averaging both kind of markers but still significantly higher than those we obtained from our Apulian pubescent oak data-set.

The only available analyses of genetic differentiation within Italian white oak populations including *Q. pubescens* s.l. carried out using EST-SSRs markers are those reported for the mixed *Q. petraea*, *Q. frainetto*, *Q. pubescens* woods of the Mount Vairano range in southern Italy (Antonecchia et al. 2015). Comparison between these analyses and our data revealed that the genetic variability of the Apulian populations is significantly lower than that recorded in Mount Vairano ($aH_e = 0.72$, k = 9.46 Ar = 9.2) as regards aH_e and Ar, and slightly higher as regards k.

Also the level of genetic variability among the *Q. pubescens* s.l. populations of the Apulia region ($F_{ST} = 0.036$) was found to be lower than the F_{ST} values found for other oak species in other parts of the world, e.g. *Q. rubra* L. ($F_{ST} = 0.080$) (Sullivan et al. 2013), *Quercus mongolica* Fisch. ex Ladeb. ($F_{ST} = 0.077$) (Ueno and Tsumura 2008), *Q. variabilis* Bl. ($F_{ST} = 0.063$) (Shi et al. 2017).

As regards gene flow, the present study showed that the Apulian *Q. pubescens* s.l. populations are characterized by a relatively high values (Nm = 6.664). Although *Q. pubescens* is a typical wind-pollinated and outcrossing species such high values are quite surprising considering the progressive contraction suffered by the Apulian oak woods in the last millennium. The value of gene flow is determined by many factors, such as the biological means of spreading via pollen and seeds, the occurrence of physical barriers among populations, population dimension and so forth; in addition, it can be facilitated by physical proximity of the populations. It is probable that the short distance separating some of the Apulian pubescent oak populations and the lack of significant mountain barriers have contributed to keep the average gene flow value high. It is not possible, at present, to establish whether the above gene-flow values are stable over time, or are the result of a contraction due to over-exploitation of forests. In studies on other oak species (Shi et al. 2017) it was established that gene-flow values can decrease significantly in a relatively short time (ten years) following the transition from primary forest to natural secondary forest. In the Apulia region the millennial exploitation of the woodlands led to the disappearance of primeval forests as early as Roman times. However, the secondary forest communities that have since replaced the primary ones show a high degree of naturalness and a rather stable floristic composition. It is conceivable therefore that the gene flow rate will remain stable over time and that the only parameter capable of negatively acting on the gene-flow rate is the spatial reduction of the population size.

The apparent relatively low level of intraspecific diversity observed for the Apulian *Q. pubescens* populations could be addressed to their marginal geographical position not only in the Italian Peninsula but also in the central Mediterranean context. In terms of biological conservation of deciduous oak forests the Apulia region is particularly exposed to environmental risks due to climate change drying effects which overlap a bioclimatic pattern dominated by the Thermo-Mediterranean thermotype the latter having in the Mediterranean maquis the main type of potential vegetation (Rivas-Martínez et al. 2004, Ladisa et al. 2012). Threats to pubescent oak woods are further increasing in many localities that are experiencing greater aridity and water deficiency due to the overexploitation of water reserves resulting from the ever increasing consumption for agricultural purposes and by tourist settlements. In the long term, the combined effect of these two factors could lead to a change in the floristic composition of the upper structural layers of the thermophilous deciduous oak forests with a progressive replacement of the pubescent oak individuals by evergreen woody species, such as *Quercus ilex* L. *Phillyrea latifolia* L., *Rhamnus alaternus* L., *Arbutus unedo* L. and *Pistacia lentiscus* L. Conversely, the high gene flow values observed in the study area seem to move in the opposite direction and might positively contribute to preventing possible activations of genetic drift. Yet the lack of bottleneck effects in all investigated populations implies a certain degree of resilience despite the relatively small areas covered by the single oak populations.

As regards possible taxonomic implications arising from this study, the results of STRUCTURE Bayesian clustering showed that the populations of Apulian pubescent oaks could not be divided into groups. None of the populations investigated displayed significant differences in their genetic composition. However, the highest percentage of the possible "putative hybrids" that we simply named "off-types", was found in Pop13, which also showed the highest percentage of private alleles. Pop13 is located within a flat sub-humid area in the western side of the Murgian plateau in the municipality of Laterza, and lies in spatial contact with a small stand of *Q. robur* wood (the only known site of *Q. robur* currently recorded for the Apulia region). It is conceivable that in this area *Q. robur* was much more abundant in the past and that both climate change and the millennial work of deforestation carried out by the local populations, has led to its current extreme impoverishment. It is very probable that during the climatic oscillations that took place during the Quaternary, environmental situations favorable to the development of mixed forests of *Q. robur* and *Q. pubescens* s.l. were created several times with consequent hybridization or introgression between these two species. In fact, a possible key of interpretation of genetic structure of the *Q. pubescens* Apulian populations cannot fail to consider some physiographic and geographical features of this region such as the lack of sizeable mountain systems and its deep wedges jutting into the Adriatic Sea. These features led to the Apulian Peninsula experiencing the effects of quaternary glaciations only mildly and allowed some parts of it to work as glacial refuges for the thermophilous oak forests during the Quaternary cold periods, when most of the Italian Peninsula was covered by steppic grasslands (Follieri et al. 1988). The occurrence of restricted areas where the climate forced the various species of white oaks to coexist for long (cold) periods may have favored the generation of hybrids. The innumerable past hybridization or introgression events, which led to the current high morphological variability of *Q. pubescens* s.l., may also have played a role in enlarging the gene flow of the Apulian populations. White oaks commonly live in sympatry, making them particularly suitable for gene exchange and production of hybrid individuals (Rushton 1993, Williams et al. 2001, Lepais and Gerber 2011). According to Burger (1975), applying the biological concept of species to the genus *Quercus* would cause an upheaval of the nomenclature, since the binomial species would then not correspond to the biological one. This makes the systematics of this genus extremely susceptible to disagreement among botanists and the hope of arriving at the proposal of a largely shared and possibly unambiguous taxonomic framework (in particular for the south-European white oaks) is something not soon to be realized. The problematic classification of pubescent oaks in the Apulian Peninsula and in the entire Italian Peninsula is perfectly evidenced by the discrepancy that emerges when comparing the taxonomy of the genus *Quercus* as reported in latest versions of Flora d'Italia (Pignatti et al. 2017) and in the Checklist of the Italian vascular flora (Bartolucci et al. 2018). Although both works are worthy of the utmost respect and consideration, they show enormous differences in both the numbers and the names of the taxa considered good species for the same taxonomic group (white oaks). Actually, based on what emerges from our work, the high taxonomic differentiation among the Italian pubescent oaks as reported in most of the Italian floras and checklists does not seem to have much foundation (see also Di Pietro et al. 2012) or at least does not seem to have it with regard to the pubescent oaks of Apulian Peninsula. In fact, our results established that it was not possible to identify genetic clusters among the pubescent oak populations of the Apulian region basing on 11 highly polymorphic markers as it was not possible to identify morphological clusters basing in a previous biometric study on the morphological traits of leaves and fruits on the same set of specimens (Di Pietro et al. 2016).

Summarizing, the presence of more than one taxon at species rank belonging to *Q. pubescens* s.l. (e.g., *Q. virgiliana*, *Q. dalechampii*, *Q. amplifolia*, etc.) is not confirmed (at least at present) for the Apulia region. At the same time, the phytosociological frameworks that are currently supposed to classify different types of pubescent oak forests (phytosociological associations), dominated by different pubescent oak species and including up to five different species of pubescent oaks in the dominant tree layer, need at the very least to be reconsidered. Obviously we do not consider this work to be a solution to all the problems concerning the taxonomy of white pubescent oaks, not even to some of them of topical relevance in south-eastern Europe, such as the eternal debate on the dualism between *Q. pubescens* and *Q. virgiliana* (cf. Borazan and Babaç 2003, Škvorc et al. 2005, Franjić et al. 2006, Jerše and Batič 2007, Trinajstić 2007, Enescu et al. 2013). We simply believe that this work represents a small step forward, a substantial contribution, albeit on a regional scale, to being able to arrive in the near future at a possible solution of the problem on a global scale. Finally, it is worth emphasizing, that all the taxonomical and phytosociological considerations mentioned in this paragraph should not be viewed as useful only for updating floras, checklists or vegetation maps. In fact, they have direct implications for the application of European Policies for Nature conservation. It is only necessary to recall the 92/43/EEC European directive where the identification of some of the forest habitats listed in the Manual of Interpretation (e.g., 91H0* "Pannonian woods with *Quercus pubescens*", 91AA* "Eastern white oak woods", 91M0 "Pannonian-Balkanic turkey oak-sessile oak forests", 91Y0 "Dacian oak and hornbeam forests"), is also based on the occurrence of species such as *Q. virgiliana* and *Q. dalechampii*.

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The dominance of a competitive grass mediates the effects of small-scale karst topography on plant species richness in a montane grassland

Goffredo Filibeck*[1], Marta G. Sperandii[2], Luca Bragazza[3], Alessandro Bricca[4], Stefano Chelli[4], Simona Maccherini[5], Camilla Wellstein[6], Antonio L. Conte[7], Marta Di Donatantonio[8], T'ai G.W. Forte[9], Lorenzo Lazzaro[10], Tania Macchiavelli[8], Samuele Maestri[1], Roberta Marchesini[11], Michela Marignani[12], Gabriele Midolo[6], Ludovica Oddi[13], Leonardo Rosati[14], Giulia Silan[15], Laura Cancellieri[1]

🖂 Corresponding author, Goffredo Filibeck: filibeck@unitus.it.
ORCID: https://orcid.org/0000-0002-4187-9467

[1] Dipartimento di Scienze Agrarie e Forestali, Università della Tuscia, 01100 Viterbo, Italy.
[2] Dipartimento di Scienze, Università Roma Tre, 00146 Roma, Italy.
[3] Agroscope, CH-1260 Nyon, Switzerland.
[4] School of Biosciences and Veterinary Medicine, Plant Diversity and Ecosystems Management Unit, University of Camerino, 62032 Camerino, Italy.
[5] Department of Life Sciences, University of Siena, Via P.A. Mattioli 4, 53100 Siena, Italy.
[5] Faculty of Science and Technology, Free University of Bozen-Bolzano, 39100 Bolzano, Italy.
[7] Department of Bioscience and Territory, University of Molise, 86090 Pesche (IS) Italy.
[8] Scuola di Scienze Matematiche, Fisiche e Naturali, Università degli Studi di Firenze, 50134 Firenze, Italy.
[9] Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università di Parma, 43124 Parma, Italy.
[10] Department of Biology, University of Firenze, Via G. La Pira 4, 50121 Firenze, Italy.
[11] Dipartimento Scienze della Vita e Biotecnologie, Università di Ferrara, 44121 Ferrara, Italy.
[12] Dipartimento di Scienze della Vita e dell'Ambiente, Università di Cagliari, 09123 Cagliari.
[13] Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino, 10124 Torino, Italy.
[14] Scuola di Scienze Agrarie Forestali Alimentari e Ambientali, Università della Basilicata, 85100 Potenza, Italy.
[15] Dipartimento di Scienze Ambientali, Informatica e Statistica, Università Ca' Foscari Venezia, 30172 Venezia, Italy.

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This PhD thesis is a contribution at the study of the biosystematic relationships among some doubtful taxa of the European pubescent White Oaks in southern Italy. This group includes Q. *pubescens* Willd., Q. *virgiliana* Ten., Q. *dalechampii* Ten., Q. *leptobalana* Guss., Q. *amplifolia* Guss., Q. *congesta* C.Presl. and Q. *ichnusae* Mossa, Bacch. & Brullo. These taxa share a large amount of morphological and ecological features and often are found in the same geographic area. The identification of these taxa and the assessment of their taxonomic rank are long-lasting problems which are still constantly under debate among the European taxonomists.

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