A peer-reviewed version of this preprint was published in PeerJ on 17 October 2018.

View the peer-reviewed version (peerj.com/articles/5793), which is the preferred citable publication unless you specifically need to cite this preprint.

Simeone MC, Cardoni S, Piredda R, Imperatori F, Avishai M, Grimm GW, Denk T. 2018. Comparative systematics and phylogeography of *Quercus* Section *Cerris* in western Eurasia: inferences from plastid and nuclear DNA variation. PeerJ 6:e5793 https://doi.org/10.7717/peerj.5793

Comparative systematics and phylogeography of *Quercus* Section *Cerris* in western Eurasia: inferences from plastid and nuclear DNA variation

Marco Cosimo Simeone [Corresp., 1], **Simone Cardoni** [1], **Roberta Piredda** [2], **Francesca Imperatori** [1], **Michael Avishai** [3], **Guido W Grimm** [4], **Thomas Denk** [5]

[1] Department of Agricultural and Forestry Science (DAFNE), Università degli Studi della Tuscia, Viterbo, Italy

[2] Stazione Zoologica Anton Dohrn, Napoli, Italy

[3] Jerusalem Botanical Gardens, Hebrew University of Jerusalem, Jerusalem, Israel

[4] Orleans, France

[5] Department of Palaeobiology, Swedish Museum of Natural History, Stockholm, Sweden

Corresponding Author: Marco Cosimo Simeone
Email address: mcsimeone@unitus.it

Oaks (*Quercus*) comprise more than 400 species worldwide and centres of diversity for most sections lie in the Americas and East/Southeast Asia. The only exception is the Eurasian Sect. *Cerris* that comprises 15 species, a dozen of which are confined to western Eurasia. This section has not been comprehensively studied using molecular tools. Here, we assess species diversity and reconstruct a first comprehensive taxonomic scheme of western Eurasian members of Sect. *Cerris* using plastid (*trnH-psbA*) and nuclear (5S-IGS) DNA variation with a dense intra-specific and geographic sampling. Chloroplast haplotypes primarily reflected geographic patterns of species coevolution within Sect. *Cerris* and its sister section *Ilex*. We identified two widespread and ancestral haplotypes, and less common, locally restricted, derived variants. Signatures shared with Mediterranean species of Sect. *Ilex*, but not with the East Asian *Cerris* siblings, suggest that the western Eurasian lineage came into contact with *Ilex* only after the first (early Oligocene) members of Sect. *Cerris* in Northeast Asia had begun to radiate and move westwards. Nuclear 5S-IGS diversification patterns were more efficient for establishing a molecular-taxonomic framework and to reveal hybridization and reticulation processes. Four main evolutionary lineages were identified. The first lineage comprises *Q. libani*, *Q. trojana* and *Q. afares* and appears to be closest to the root of Sect. *Cerris*. These taxa are morphologically most similar to the East Asian species of *Cerris*, and to both Oligocene and Miocene fossils of East Asia and Miocene fossils of western Eurasia. The second lineage is mainly composed of the widespread *Q. cerris* and the narrow endemic species *Q. castaneifolia, Q. look*, and *Q. euboica*. The third lineage comprises three Near East species (*Q. brantii*, *Q. ithaburensis* and *Q. macrolepis*), well adapted to continental climates with cold winters. The forth

lineage appears the most derived and comprises *Q. suber,* the cork oak, and *Q. crenata*. *Quercus cerris* and *Q. trojana* displayed exceptional levels of variation; *Q. macrolepis* and *Q. euboica,* previously treated as subspecies of *Q. ithaburensis and Q. trojana,* likely deserve an independent species status. A trend towards inter-specific crosses was detected in several taxa; however, we found no clear evidence of a hybrid origin of *Q. afares* and *Q. crenata*, as currently assumed. Phylogeographic inferences on the origin and diversification of *Quercus* Sect. *Cerris* are provided to fill an important gap in the knowledge of oak diversity and evolution.

Title: Comparative systematics and phylogeography of *Quercus* Section *Cerris* in western Eurasia: inferences from plastid and nuclear DNA variation

Authors: Marco C. Simeone[1*], Simone Cardoni[1], Roberta Piredda[2], Francesca Imperatori[1], Michael Avishai[3], Guido W. Grimm[4], Thomas Denk[5]

Affiliations:

[1]Dipartimento di Scienze Agrarie e Forestali (DAFNE), Università degli studi della Tuscia, via S. Camillo de' Lellis, 01100 Viterbo, Italy

[2]Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy

[3]Jerusalem Botanical Gardens, Hebrew University, Givat Ram, 91904 Jerusalem, Israel

[4]Unaffiliated, 45100 Orléans, France

[5]Department of Palaeobiology, Swedish Museum of Natural History, Box 50007, 10405 Stockholm, Sweden

Corresponding author:

*Marco Cosimo Simeone

DAFNE, università degli studi della Tuscia, via S. Camillo de' Lellis, 01100 Viterbo, Italy

Tel. +39 0761 357352

Fax +39 0761 357350

Email: mcsimeone@unitus.it

Abstract

Oaks (*Quercus*) comprise more than 400 species worldwide and centres of diversity for most sections lie in the Americas and East/Southeast Asia. The only exception is the Eurasian Sect. *Cerris* that comprises 15 species, a dozen of which are confined to western Eurasia. This section has not been comprehensively studied using molecular tools. Here, we assess species diversity and reconstruct a first comprehensive taxonomic scheme of western Eurasian members of Sect. *Cerris* using plastid (*trnH-psbA*) and nuclear (5S-IGS) DNA variation with a dense intra-specific and geographic sampling. Chloroplast haplotypes primarily reflected geographic patterns of species coevolution within Sect. *Cerris* and its sister section *Ilex*. We identified two widespread and ancestral haplotypes, and less common, locally restricted, derived variants. Signatures shared with Mediterranean species of Sect. *Ilex*, but not with the East Asian *Cerris* siblings, suggest that the western Eurasian lineage came into contact with *Ilex* only after the first (early Oligocene) members of Sect. *Cerris* in Northeast Asia had begun to radiate and move westwards. Nuclear 5S-IGS diversification patterns were more efficient for establishing a molecular-taxonomic framework and to reveal hybridization and reticulation processes. Four main evolutionary lineages were identified. The first lineage comprises *Q. libani*, *Q. trojana* and *Q. afares* and appears to be closest to the root of Sect. *Cerris*. These taxa are morphologically most similar to the East Asian species of *Cerris*, and to both Oligocene and Miocene fossils of East Asia and Miocene fossils of western Eurasia. The second lineage is mainly composed of the widespread *Q. cerris* and the narrow endemic species *Q. castaneifolia, Q. look*, and *Q. euboica*. The third lineage comprises three Near East species (*Q. brantii*, *Q. ithaburensis* and *Q. macrolepis*), well adapted to continental climates with cold winters. The forth lineage appears the most derived and comprises *Q. suber,* the cork oak, and *Q. crenata*. *Quercus cerris* and *Q. trojana* displayed exceptional levels of variation; *Q. macrolepis* and *Q. euboica,* previously treated as subspecies of *Q. ithaburensis and Q. trojana,* likely deserve an independent species status. A trend towards inter-specific crosses was detected in several taxa; however, we found no clear evidence of a hybrid origin of *Q. afares* and *Q. crenata*, as currently assumed. Phylogeographic inferences on the origin and diversification of *Quercus* Sect. *Cerris* are provided to fill an important gap in the knowledge of oak diversity and evolution.

53 **Keywords** *Quercus*, Section *Cerris*, Plastid DNA, Nuclear Ribosomal 5S-IGS, Western Eurasia,
54 Evolution

Introduction

Studies on the genetic diversity of forest species across their distributional ranges are relevant for genetic resource inventories and devising conservation strategies (Pautasso, 2009). Comparative phylogeographic studies may further reveal complex spatial variation patterns within groups of closely related species (sibling lineages, species aggregates), shaped by partly antagonistic evolutionary and ecological processes. The detailed genetic information can be used to address taxonomic questions, assist biodiversity surveys, and implement species conservation and future landscape management strategies (Barak et al., 2016).

Oaks (*Quercus* L.) are a worst case and, at the same time, an ideal model for comparative phylogeographic studies. They are common, often (co-)dominant vegetation elements and include several widely distributed and ecologically diverse species (Camus, 1936–54; Schwarz, 1936). Oaks have a strong potential for ecological adaptation, accompanied by substantial leaf morphological variability, and high potential for introgression and reticulate evolution (e.g. Burger, 1975; Van Valen, 1976; Petit et al., 2004; McVay et al., 2017). Therefore, regional estimates about the number of oak species have been strongly deviating (see e.g. IPNI, 2017). *Quercus* currently comprises more than 400 species occurring throughout the Northern Hemisphere and including several species in the tropics (Govaerts & Frodin, 1998; Denk et al., 2017). Oaks extend into the boreal and subalpine zones (Walter & Breckle, 1983–1991; Schroeder, 1998) including continental, cold-temperate settings (e.g. Fang et al., 2009). The main lineages recognized based on (pollen) morphology and molecular markers have recently been formalized as two subgenera with eight sections (Denk et al., 2017). The predominantly Nearctic Subgenus *Quercus* includes sections *Lobatae* (Americas), *Protobalanus* (western North America), *Ponticae* (two disjunct species in southwestern Georgia/ northeastern Turkey and northern California/ southwestern Oregon), *Virentes* (southeastern U.S. into Mesoamerica), and *Quercus* (with most species in North America and about 30 species in Eurasia). The Palearctic-Indomalayan Subgenus *Cerris* includes sections *Cyclobalanopsis* (East Asia), *Ilex* and *Cerris* (across Eurasia). For some of these groups, detailed infragroup phylogenies and assessment of main biogeographic patterns have recently been published (e.g. Hipp et al., 2014: North American Sect. *Quercus*; Cavender-Bares et al., 2015: Sect. *Virentes*; Vitelli et al., 2017: western Eurasian members of Sect. *Ilex;* Deng et al., 2018: Sect. *Cyclobalanopsis*).

Section *Cerris* (*Cerris* oaks) currently comprises 12 or 13 species (Table 1) occurring from the Atlantic coasts of the Iberian Peninsula and Morocco to Japan. They thrive under a variety of climates (Köppen-Geiger climate types): cold steppe (*BSk*) and warm temperate or snow climates with different precipitation regimes (*Cs*, *Cf*, *Cw*, *Ds*, *Df*, *Dw*; Kottek et al., 2006; Peel et al., 2007; Rubel et al., 2016). These oaks are deciduous or semi-evergreen (i.e. leaves are kept for about 12–18 months) trees up to 30 m tall, characterized by pollen with scattered verrucate ornamentation, imbricate, recurved and elongated cupule scales, tomentose endocarp, and pointed, elongated styles; their leaves are generally toothed or lobed and usually with a mucronate apex (Denk et al., 2017). Based on the fossil record and molecular differentiation patterns it has been suggested that Sect. *Cerris* evolved from Sect. *Ilex*, possibly in Europe during the Miocene (Denk & Grimm, 2010; Simeone et al., 2016). However, this scenario needs to be revised as unambiguous fossils of Sect. *Cerris* are now known from early Oligocene deposits of northeastern Russia (Russian Far East), and Sect. *Ilex* appears to have been present in middle Eocene strata of southern China (Denk et al., 2017).

At present, the highest species diversity of Sect. *Cerris* lies in western Eurasia, with eight commonly accepted species (Goevarts & Frodin, 1998) and some additional taxa (*Q. crenata, Q. look, Q. trojana* subsp. *euboica, Q. ithaburensis* subsp. *macrolepis*) of disputed or unresolved status. The distribution areas of the *Cerris* oaks vary substantially in size and in the degree of contact with other species of the same section and the sister section *Ilex*. The Qinghai-Tibet Plateau and the Himalayan front-hills separate the East Asian and the western Eurasian taxa, with the central and eastern Mediterranean region bearing most of the group's diversity (Anatolia and Levant to S. Italy and N.E. Algeria, 8 species), decreasing westward (Iberian Peninsula and Morocco, one species) and eastward (Iran/ Iraq, three species; Browicz & Zieliński, 1982). Two species have broad distributions (*Q. suber,* the 'Cork Oak', partly due to cultivation, and *Q. cerris*), and three are geographically extremely limited (*Q. afares*, *Q. castaneifolia, Q. look*). A hybrid origin has been postulated for *Q. crenata* and *Q. afares* (Mir et al., 2006; Conte et al., 2007). Other occasional infra-sectional hybrids have also been described as morphologically intermediates (see Menitsky, 2005), while *Q. suber* shows interfertility with a member of Sect. *Ilex* (*Q. ilex*; Burgarella et al., 2009).

Detailed phylogeographic inferences based on extensive sampling and plastid DNA sequence analyses are so far available only for two East Asian species of Sect. *Cerris* thriving in temperate

and subtropical broad-leaved forests in eastern Asia, *Q. acutissima* and *Q. variabilis* (Chen et al., 2012; Zhang et al., 2015). In both cases, high genetic diversity but weak phylogeographic structures were found, explained with recent (Pleistocene) speciation and post-glacial re-expansion of the lineages. The two most widespread western Eurasian species (*Q. suber* and *Q. cerris*) were studied using plastid microsatellite variation (Magri et al., 2007; Bagnoli et al., 2016). Geographic-structured gene pools were detected, and attributed to the Oligocene (*Q. suber*) and Pleistocene (*Q. cerris*), respectively. Local investigations focussing on conservation were conducted on *Q. trojana* in Italy and *Q. libani* in Iran using nuclear microsatellites (Khadivi-Khub et al., 2015; Carabeo et al., 2017). Finally, species of the entire section were included in DNA barcoding projects and studies on molecular macroevolution (e.g. Denk & Grimm, 2010; Simeone et al., 2013; 2016), but relied on a limited number of individuals. Hence, a comprehensive assessment of the taxonomy, diversity and evolutionary history of the whole section *Cerris* is currently not available.

At present, firm species delineation and phylogenetic inferences using nuclear sequence data are difficult in *Quercus*, mostly due to the limited availability of genes and regions with sufficient levels of variation, especially when closely related species are involved (Muir et al., 2001; Oh & Manos, 2008; Hubert et al., 2014). Technical obstacles such as reliable PCR amplification, sequence quality, unrepresentative intra- and inter-specific sampling constitute additional obstacles (Manos et al., 2001; Bellarosa et al., 2005; Chen et al., 2017). Phylogenomic (RADseq) data can provide well-resolved within-lineage relationships (Hipp et al., 2014, 2017; Cavender-Bares et al., 2015); however, the complexity and costs of the technique prevent investigation of large samples. Denk & Grimm (2010) tested the efficacy of the nuclear ribosomal 5S rDNA intergenic spacer to resolve relationships of western Eurasian oaks. Especially in Sect. *Cerris*, this region allowed differentiation at the species level, in contrast to the internal spacers of the 35S rDNA, the ITS1 and ITS2 (i.e. the most widely used nuclear marker for biotaxonomic inferences and barcoding; see Yang et al., 2017). Being potentially affected by incomplete lineage sorting, intra-array recombination and intragenomic competition, this marker requires a special analysis framework (cloning and *host-associate* analysis; cf. Göker & Grimm 2008) but enables tracking of reticulate evolutionary signatures.

On the other hand, being largely controlled by provenance and decoupled from speciation, plastid data are important to trace the radiation of a lineage in space and time (Pham et al., 2017).

Two recent studies on the Mediterranean members of Sect. *Ilex* (Simeone et al., 2016; Vitelli et al. 2017) found three main plastid haplotype groups, with a distinct geographic distribution and phylogenetic quality: (1) 'Euro-Med' comprising the most distinct haplotypes dominating in the western Mediterranean, a plastid lineage that diverged before the radiation of plastid pools in Subgenus *Cerris*, (2) 'WAHEA', distributed from Anatolia/Levant to East Asia, and (3) 'Cerris-Ilex', centred on the Aegean Sea and shared with co-occurring members of the Sect. *Cerris*. 'WAHEA' and 'Cerris-Ilex' haplotypes constitute sister lineages. Haplotype variation of the *trnH-psbA* intergenic spacer was determinant for the phylogeographic inferences; this marker also showed the highest variation rate among several plastid regions in 35 Chinese oak species (Yang et al., 2017), and in the comprehensively studied *Q. acutissima* and *Q. variabilis* (Zhang et al., 2015; Chen et al., 2012).

Clearly, full comprehension of the drivers of speciation of Sect. *Cerris* requires information from both genomes based on extensive geographic and taxonomic collections. In this work, we investigated 5S-IGS and *trnH-psbA* molecular diversity in western Eurasian Sect. *Cerris* using a comprehensive intra- and inter-specific sampling. Our objectives were: (i) to assess species coherence and delimitation, (ii) to infer inter-species relationships, (iii) to gain insight into the origin and diversification of the group.

Materials and methods

Plant material and DNA sequencing

We combined previously studied (Denk & Grimm, 2010) and new material to develop a sampling design of 221 individuals covering the whole distribution range of *Quercus* Sect. *Cerris* in western Eurasia (Supplementary File S1); some individuals with perplexing morphology were labelled as presumed hybrids, based on the intermediacy of characters. DNA was extracted from silica gel-dried leaf samples with the DNeasy Plant minikit, following the manufacturer's instructions. The *trnH-psbA* intergenic spacer was amplified and sequenced following Simeone et al. (2016). The nuclear ribosomal 5S intergenic spacer (5S-IGS) was amplified with the primer pair 5S14a and 5S15 (Volkov et al., 2001; Denk & Grimm, 2010). Individual PCR fragments were ligated into a pGEM-T easy vector (Promega). The ligation mixtures, purified with the Illustra GFX PCR DNA Purification kit (GE Healthcare), were used

to transform *E. coli* strain XL1-Blue electroporation-competent cells (recA1, endA1, gyrA96, thi-1, hsdR17, supE44, relA1, lac, [F' proAB, lacIqZΔM15, Tn10 (tetr)]). The positive clones, selected on LB/Ampicillin plates, were identified by colony PCR using the amplification primers. Five to ten recombinant clones per individual were sequenced with the vector-specific universal primers (SP6/T7) at LGC Genomics (Augsburg, Germany). The GenBank *trnH-psbA* sequences of several East Asian members of Sect. *Ilex* (*Q. baloot*, *Q. floribunda*, *Q. phylliraeoides*, *Q. semecarpifolia*, *Q. baroni*, *Q. dolicholepis*, *Q. spinosa*), East Asian species of Sect. *Cerris* (*Q. acutissima, Q. variabilis*) and further sequence accessions of the investigated group were included in the analyses. In addition, all 5S IGS sequences of *Quercus* Sect. *Cerris* from Denk and Grimm (2010) were included in the final dataset, and the sequences of *Q. baloot* and *Q. floribunda* were used as outgroups (all GenBank accession numbers reported in File S1).

Data analyses

Eye-checked electropherograms were aligned in MEGA7 (Kumar et al., 2016). Highly dissimilar clone sequences showing no BLAST match with the targeted regions (Altschul et al. 1990) were filtered. Final multiple alignments were obtained with ClustalW 1.81 (Thompson et al., 1994) and checked by eye. The diversity of the investigated regions was evaluated with MEGA7 and DnaSP5.1 (Librado & Rozas, 2009). Median-joining (MJ) haplotype networks for the *trnH-psbA* region were inferred with Network 4.6.1.1 (http://www.fluxus-engineering.com/), treating gaps as 5th state. The MJ algorithm was invoked with default parameters (equal weight of transversion/transition), in order to handle large datasets and multistate characters.

After removal of identical clones, the total 5S-IGS sequences were used to build a Maximum likelihood (ML) tree with RAxML v8.2 (Stamatakis, 2014) using the in-built GTR+Γ model with the 'extended majority-rule consensus' criterion as bootstopping option (Pattengale et al., 2009). To infer inter-individual relationships, we applied the approach described by Göker & Grimm (2008) that allows transformation of data matrices of 'associates' (here: cloned sequences) into 'hosts' (here: individuals). The program G2CEF (available at http://www.goeker.org/mg/distance/) was used to transform the primary character matrix ('associates', total cloned sequences) into a character consensus matrix of the individuals ('hosts') using an association file defining the list of clone sequences belonging to the same individual. The uncorrected pairwise distances of the primary character matrix ('associates', total

cloned sequences) was calculated and used as input to the program PBC (Göker & Grimm, 2008). This program allows transforming the primary inter-clone pairwise distance matrix into inter-individual distances matrices using different flavours, of which the 'Phylogenetic Bray-Curtis' (PBC) transformation performed best in the original study that compared data sets with similar properties than our data set. Here, we applied three of the distance transformations tested by Göker & Grimm (2008), in addition to PBC distances (option -b) also the minimum (MIN; -i) and average (AVG; -a) inter-individual clonal distances. AVG, MIN and PBC distance matrices were generated setting different minimum number of associates per host (-m option); m=4 (the number of cloned sequences obtained in most individuals) was then used to infer a phylogenetic network using the Neighbour-Net (NN) algorithm (Bryant & Moulton, 2004) implemented in SplitsTree4 (Huson & Bryant, 2006).

Results

In total, 221 individuals effectively covering the taxonomic range of western Eurasian Sect. *Cerris* were analysed (Table 1, File S1). Sequence quality was high for both marker regions and unambiguous electropherograms were obtained for about 90% of the investigated samples. The primary data matrixes comprised 207 plastid (*trnH-psbA*) and 856 nuclear (5S-IGS) sequence accessions. The nuclear data (192 total individuals) included 651 newly sequenced clones and 205 accessions from Denk & Grimm (2010). Ten *Q. baloot/ Q. floribunda* sequences, used here as outgroups (cf. Denk & Grimm, 2010), extended the final dataset to 866 sequenced clones. Individual sequences recovered from positive 5S-IGS clones varied from one (four samples) to 10, with most samples represented by four sequences (70 samples), followed by five and three sequences (44 and 35 samples, respectively). Multiple alignments of both marker regions were straigthforward. A 34-bp inversion occurring in the *trnH-psbA* region of 14 samples was replaced with its reverse-complementary sequence and a binary character was inserted to keep record of it.

Plastid *trnH-psbA* diversity and biogeography

After removing the 34-bp inversion, the *trnH-psbA* marker showed pairwise uncorrected *p*-distances ranging between zero and 0.008 (Table 2). The highest intra-specific distance (0.006)

was found in *Q. suber*; four species showed similar values (0.002; *Q. cerris*, *Q. ithaburensis*, *Q. trojana*, *Q. libani*), while the marker variation in the remaining taxa converged to zero.

The total matrix was 503-bp characters long, including several indels (1–8 bp) and six polymorphic sites resulting in twelve haplotypes (labelled H1-H12) with a medium overall diversity ($h = 0.515$). Haplotype H1 hit 100% sequence identity with three non-representative individuals assigned to East Asian species of Sect. *Ilex* in Genebank (haplotype list, occurrence and gene bank matches shown as Files S1 and S2). It was the most common haplotype, occurring in 68.6% of individuals and all taxa except *Q. brantii*, *Q. look,* and *Q. ithaburensis* subsp. *ithaburensis* (henceforth *Q. ithaburensis*). Haplotypes H2, H5–H7 and H11 showed 100% sequence identity with Mediterranean members of Sect. *Ilex* (Simeone et al. 2016; Vitelli et al. 2017). H2 is the second most frequent haplotype, found in 10.6% of *Q. cerris*, *Q. trojana, Q.* ithaburensis subsp. *macrolepis* (henceforth *Q. macrolepis*) samples from Turkey, the Balkans and Italy; H5-H7 were found in *Q. brantii*, *Q. cerris* and *Q. macrolepis* from Turkey, Iran, and Israel. They were all shared with *Q. coccifera* and *Q. ilex* of the Aegean 'Cerris-Ilex' lineage. Haplotype H11 found in Iberian samples *Q. suber* was shared with *Q. ilex* of the 'Euro-Med' lineage. Rare haplotypes restricted to a single species were H7 (one accession of *Q. cerris*), H8 (three accessions of *Q. libani*; new 'Cerris-Ilex' subtypes) and H11/H12 (8 accessions of *Q. suber*; 'Euro-Med' types); all other haplotypes were shared by more than one species of Sect. *Cerris*. *Quercus cerris*, the most widespread and ecologically diverse species of Sect. *Cerris*, showed the highest number of haplotypes (8), followed by *Q. brantii* (5) and *Q. macrolepis* (4). In this latter species, haplotype H6, exclusively found in *Q. brantii*, was also found in a suspected hybrid *Q. macrolepis* x *Q. brantii* (sample ml27). All samples of *Q. ithaburensis* exhibited one single haplotype (H9). The geographically (more) restricted taxa *Q. afares*, *Q. castaneifolia, Q. crenata* and *Q. trojana* subsp. *euboica* (henceforth: *Q. euboica*) showed only the most frequent and widespread haplotype (H1).

In comparison (Table 2), the two East Asian members of Sect. *Cerris* (*Q. acutissima* and *Q. variabilis*) displayed a higher variation at the *trnH-psbA* locus, although mostly due to indels. A higher number of haplotypes was found in these species; none of them was shared with any species of Sect. *Ilex* available in gene banks (identity range: 93–99% with *Q. baroni*, *Q. dolicholepis* and/or *Q. spinosa*), and only one haplotype was shared between the two species.

No shared parsimony informative characters (PICs) were found in the included East Asian samples. In contrast, three PICs were exclusively shared by haplotypes H11 and H12 (*Q. suber* from Iberian Peninsula, North Morocco). One further PIC separated H9, including all individuals of *Q. ithaburensis*, two *Q. look*, two Israelian and one Italian *Q. cerris* individuals. A single PIC also defined H4, including three co-occurring *Q. trojana* and *Q. macrolepis* accessions from the same locality in western Turkey, and another PIC was limited to two *Q. cerris* and *Q. libani* accessions from southern Turkey, corresponding to H3. Table 3 shows that the highest mean intragroup divergence in the West Eurasian dataset was found in *Q. suber* and *Q. libani*. The haplotypes of *Q. suber* and *Q. look* displayed the highest mean divergence from all other species. *Quercus variabilis* appeared more similar to its western Eurasian counterparts, while *Q. acutissima* was highly divergent.

The haplotype network shown in Fig. 1 evidences the general coherence of the 'Cerris-Ilex' lineage, which collects haplotypes typical for the western Eurasian members of Sect. *Cerris*, clearly distinct from the haplotypes found in East Asian members of Sect. *Cerris* and haplotypes H11–H12 (>5 mutations separating each lineage); this latter represents a unique, early diverged plastid lineage, most frequent in the western Mediterranean populations of Sect. *Ilex* (Simeone et al., 2016; Vitelli et al., 2017). Based on the relative number of mutations (1–5) separating each haplotype, the 'Cerris-Ilex' lineage can be further subdivided into two groups: (L1) a group of potentially primitive (non-derived) haplotypes (H1–H4) including the most common haplotypes (H1, H2) and still close to haplotypes found in the north-easternmost species of Sect. *Ilex* (*Q. phylliraeoides*), the plastid sister lineage of 'Cerris-Ilex' (Simeone et al., 2016); (L2) a group of derived haplotypes (H5–H10). Haplotypes not shared with Sect. *Ilex* (H3–H4, H8–H10, H12) are derivates of the 'Cerris-Ilex' and 'Euro-Med' main types (H1–H2, H5-H7, H11), shared by both sections in the Aegean and the western Mediterranean regions. As shown in Fig. 2a-b, plastid diversity is largely decoupled from species identity, and related to geography; the least derived haplotypes within the 'Cerris-Ilex' lineage (H1 and H2) occur across the whole distribution range of the investigated group, except the Levant and the western Mediterranean (H2). All other haplotypes are more circumscribed, concentrated in Anatolia (H3–H5, H7–H8), the Levant (H6, H9–H10; the latter two showing single occurrences in Italy), and Iberian Peninsula + Morocco (H11-H12).

Nuclear 5S rDNA diversity and species phylogeny

In contrast to *trnH-psbA*, 5S-IGS sequence variation appeared generally correlated with the taxonomy of the studied individuals, and allowed inferences on potential reticulation and inter-species relationships within the western Eurasian members of Sect. *Cerris*. The 5S-IGS clones varied greatly in sequence features and length (the multiple alignment of the cloned sequences can be viewed in the Online Supplementary Archive at the journal's homepage). For instance, all *Q. brantii* clones displayed an intra-specific $(ATTT)_{1\text{-}7}$ simple sequence repeat (SSR) variation. In all the other species, this motif was either absent (replaced by a 5–12 bp long poly-T) or consisting of 1–2 repeats, with the exception of two clones of the suspected hybrid *Q. macrolepis* x *Q. brantii* (individual ml27) that showed 4–5 repetitions. Two clones of *Q. brantii* (sample br02; C. Turkey) shared a 4-bp insertion with several clones of sympatric *Q. macrolepis* individuals (ml20–ml22). Three *Q. libani* individuals (li02, 03, 04; S. and E. Turkey) displayed a long indel (ca. 100 bp) in (nearly) all clones ('short *libani* variant'; cf. Denk & Grimm, 2010). The extended sample revealed that the 'short *libani* variant' is not exclusive to *Q. libani* but is rarely found also in *Q. cerris* (clones ce2104 and ce4704; Italy, W. Turkey) and *Q. trojana* (three clones of individual tj33, S. Turkey); the latter, however, is another suspected hybrid (*Q. trojana* x *Q. libani*).

Two other deletions were detected in the same region of the 'short *libani* variant'. One (22 bp) was shared by single clones of two *Q. cerris* individuals (ce18, ce22; S.W. and W. Turkey), four clones of a *Q. look* individual (lk2; Israel) and two clones of *Q. trojana* (individual tj40; S. Turkey). The second (~100 bp), largely overlapping the deletion of the '*short libani variant*', but beginning a few basepairs downstream, was shared by one clone of *Q. cerris* (ce34; N. Turkey) and one clone of *Q. macrolepis* (ml26; S. Greece). An 8-bp deletion occurred exclusively in *Q. suber* and *Q. crenata*, with the exception of single clones of samples su07, su09 (N.E. and S. Spain), su37 (Croatia), su53 (S. Italy), cr02 (C. Italy), two clones of sample cr04 (Slovenia) and cr06 (N.E. Italy), and three clones of sample cr05 (Croatia). The same deletion also occurred in two clones of sample tj08, a *Q. suber* x *Q. trojana* cultivation hybrid. Further deletions (1–60 bp) were scattered along the alignment and found only in single individuals (e.g. it04, Israel; ml10, N.W. Greece). Finally, an 18-bp highly variable region was exclusively found in some clones of four co-occurring *Q. trojana* samples (tj03–05, tj16; S.C. Turkey).

The main diversity values of the investigated dataset are reported in Table 4. Identical 5S-IGS sequences typically occur in the same individual and species, and, to a lesser extent, in sympatric, different species (e.g. *Q. brantii*, *Q. cerris*, *Q. trojana*, *Q. look*; see also File S3). On the contrary, *Q. afares*, *Q. castaneifolia*, *Q. libani, Q. ithaburensis, Q. macrolepis* and *Q. euboica* showed high species coherence. *Quercus suber* and *Q. macrolepis* showed the highest number of intra-individual and intra-specifically shared clones, whereas *Q. cerris, Q. trojana* and *Q. ithaburensis* displayed the highest levels of unique variants. No variants were shared between *Q. trojana* and *Q. euboica*; *Q. suber* and *Q. crenata* (but not *Q. cerris*) shared 69 identical sequences and are the genetically most similar taxon pair. The pairwise uncorrected *p*-distance range of the total dataset was much higher than for the plastid marker (0–0.209), with highest values scored by *Q. cerris* and *Q. trojana*. The mean intra-specific molecular diversity estimated within sequence pairs (Table 5) was lowest in the two narrow endemics *Q. afares* and *Q. castaneifolia* and highest in *Q. brantii* and *Q. ithaburensis*. Across the entire dataset, *Q. brantii, Q. macrolepis* and *Q. ithaburensis* were the most diverging taxa; the least divergent were *Q. afares* and *Q. castaneifolia*. The mean divergence value between *Q. macrolepis* and *Q. ithaburensis* (0,0376), treated as subspecies of *Q. ithaburensis* in current regional floras, was similar to values detected between these taxa and the other species (e.g. *Q. afares*, *Q. brantii*, *Q. libani*). Likewise, the divergence recorded between the putative conspecific *Q. trojana* and *Q. euboica* (0.0266) was comparable to the estimates calculated between these and other taxa (e.g. *Q. afares*, *Q. cerris*, *Q. look*, *Q. libani*). The putative hybrid taxon *Q. crenata* displayed the lowest divergence (0.0197) with *Q. suber,* one of the assumed parental species, and a slightly higher estimate (but similar to the values scored with other taxa, e.g. *Q. afares*, *Q. castaneifolia, Q. look)* with *Q. cerris* (0.0266), the other putative parental species.

The clone-based ML tree rooted on *Q. baloot* and *Q. floribunda* (West-Asian members of Sect. *Ilex*) showed four main topological features (grades/clades) generally coherent with taxonomy (Fig. 3, see also File S4). These grades/clades collected to a large degree clones of (1) *Q. crenata* and *Q. suber* (resolved as proximal, weakly differentiated grade), (2) *Q. brantii, Q. ithaburensis* and *Q. macrolepis* (the most highly supported clade: BS_{ML} = 84), (3) *Q. trojana* (a large heterogenous grade), and (4) *Q. cerris* (the distal, terminal, clade with diminishing support). *Quercus libani* clones (short and normal-length variants) were present in all clades/grades except grade 1. A moderately supported clade (BS = 63) including all *Q. afares* clones was placed as

sister to the main clade including clades/grades 2 to 4; *Q. castaneifolia* clones were placed within grade 3. Clones of *Q. ithaburensis* also occurred in grade 3, *Q. brantii* and *Q. crenata* in clade 4, *Q. look* and *Q. euboica* in grade 3 and 4. A few clones of *Q. cerris* and *Q. trojana* occurred scattered across the tree (often in proximal positions).

Of the three clones sequenced from individual ml27, suspected *Q. macrolepis* x *brantii* hybrid, one was identical to another *Q. macrolepis* clone (individual ml08) and the other two clustered together with *Q. brantii*. Likewise, three and two of the five clones sequenced in sample tj08, a *Q. trojana* x *Q. suber* hybrid, clustered within the respective parental subtrees; the same applies to the five clones of the sample tj33, a supposed *Q. libani* x *Q. trojana* hybrid. Conversely, all the three clones sequenced in sample tj02, another tree determined as possible *Q. libani* x *Q. trojana* hybrid, clustered with *Q. trojana*.

The networks based on transformed 5S-IGS data (Fig. 4 based on AVG-transformed uncorrected distances; Fig. 5 based on PBC-transformed distance matrix; only individuals represented by more than four clones included) largely confirmed the earlier found intra- and inter-species relationships (Denk & Grimm, 2010; because of the amount of shared identical clones, the MIN-transformed networks are largely collapsed, but included in the Online Supporting Archive). Four clusters emerged clearly. Cluster 1, the 'oriental' lineage of Sect. *Cerris*, is the least coherent cluster and equivalent to a grade in a corresponding outgroup-rooted (*Q. baloot, Q. floribunda*) tree. This lineage included, in the AVG network, *Q. afares*, four out of five *Q. libani* individuals and about half of the *Q. trojana* individuals (Fig. 4). Its counterpart, Cluster 2, the 'occidental' lineage, accomodated all *Q. look, Q. euboica,* the remaining *Q. trojana* and *Q. libani* samples, and all but one *Q. cerris* individual (ce50; Figs 4, 5). The PBC network (Fig. 5) reveals a more gradual shift between these two clusters, with *Q. afares* splitting off with two genetically similar *Q. cerris* and *Q. libani* individuals (ce50, li05). The reason for this is that the PBC transformation has a higher chance to capture evolutionary signals (Göker & Grimm, 2008). Cluster 3 included *Q. suber* and *Q. crenata*, here, the only difference is the boxyness inflicted by the genetically intermediate individuals cr05 and tj08. Cluster 4 included the 'Vallonea' (or *Aegilops*) oaks, *Q. brantii, Q. ithaburensis* and *Q. macrolepis*, with two *Q. brantii* individuals (br02 and br03, with diverging 5S-IGS features and variants; File S4) in proximal (br02 in Figs 4, 5; br03 in Fig. 4) or off-cluster (br03 in Fig. 5) position. Thus, the basic structure of the AVG

and PBC networks and the ML tree are equivalent, but they differ in placing the outgroup taxa, and the networks refine inter-species relationships.

The AVG network (Fig. 4) captures better putative hybrids and introgrades. Strong ambiguous signals came from the hybrid *Q. trojana* x *Q. suber* (tj08), one *Q. crenata* individual (cr05, terminals in the box-like structure connecting the 'occidental', cluster 2, and *crenata-suber* lineage, cluster 3), and one *Q. ithaburensis* individual (it03, terminal in the box-like structure between clusters 2 and 4). The placement of one *Q. libani* individual (li01, inserted in cluster 2), with normal-long variants in the clone sample, and one *Q. cerris* (ce50, in cluster 1 close to the *Q. afares* subgroup; cf. Fig. 5) does not follow the general trend. Long terminal edges indicative of unique individual clone samples (combinations) are found in each cluster. Besides the outgroup *Q. baloot* and *Q. floribunda*, these samples include individuals of *Q. cerris* (ce29, 44), *Q. trojana* (tj03, 16, 24, 39, 45), *Q. suber* (su07, 29, 49), *Q. brantii* (br06), *Q. ithaburensis* (it04, 05), and *Q. macrolepis* (ml10). Some of these samples had unique deletions or highly divergent regions in their clones (e.g. it04, ml10, tj03, tj16; see above). The networks produced with individuals represented by ≥2, ≥3 (and ≥5) clones did not change this structure (File S5); they allowed inclusion of all individuals into the four clusters matching the general scheme and pinpointed a few other (possible) exceptions. *Quercus castaneifolia* (represented by two clones) and one sample of *Q. crenata* (cr04; three clones) formed part of the 'occidental' lineage, cluster 2; one sample of *Q. suber* (su09; three clones) was included in cluster 1, the 'oriental' lineage of Sect. *Cerris* (see Table 4), and sample br01 (three clones) was placed at the root of cluster 4, similarly to samples br02 and br03. The geographical distribution of the four clusters is shown in Fig. 2c.

In contrast, the PBC network (Fig. 5) provided a better basis for inferring the evolution and differentiation (speciation) processes. The 'oriental' (cluster 1) and 'occidental' lineages are clearly connected and form a continuum, with the easternbound *Q. trojana* and *Q. libani* representing a diverged, differentiated pool from which the other species and western *Q. trojana* derived. The western Mediterranean *crenata-suber* lineage is clearly different and only linked to the main pool by occasional introgression or hybridisation with nearby members of the 'occidental' lineage (in nature: *Q. cerris*). The same holds even more for the 'Vallonea' oaks (Cluster 4), which appear to have split before the remainder of western Eurasian *Cerris* (but long-branch/-edge attraction with the extreme long-edged outgroup needs to be considered). A

clear signal in the PBC network (Fig. 4) is the uniqueness of *Q. afares*, a disjunct outpost of the putative 'oriental' lineage, genetically closely related to geographically very disjunct (C./S. Anatolian) individuals of *Q. cerris* and *Q. libani* (cf. Fig. 3 showing an *Q. afares* subclade, and File S4, same tree with clones labelled).

Discussion

The western Eurasian members of Sect. *Cerris* exhibit a *trnH-psbA* diversity well comparable with the Mediterranean oaks of Sect. *Ilex* (Vitelli et al., 2017) and Fagaceae in general (Simeone et al., 2016). As discussed in Grímsson et al. (2016), the plastid genealogy in this genus is largely decoupled from species identity. Nevertheless, the strong geographic signal of plastid data provides useful information to decipher population-area relationships and taxon histories (e.g. isolation, reticulation, introgression; cf. Pham et al., 2017). Our data of the intergenic spacers of the 5S rDNA, instead, confirmed their status as most variable nuclear gene region for a large range of plants (Volkov et al., 2001; Forest et al., 2005; Lehtonen & Myllys, 2008; Denk & Grimm, 2010; Grimm & Denk, 2010). They were highly variable across the entire dataset and displayed inter-individual patterns that allowed circumscription of most of the investigated species; the intra-individual variation in the 5S-IGS further helped to recognize hybridization and infer other reticulation events such as introgression. Combined data were concordant with the known ecology and biogeography of the studied taxa.

Molecular recognition of species and species diversity in *Quercus* Section *Cerris*

Widespread species such as *Q. cerris*, *Q. suber*, and (to a lesser extent) *Q. libani* (Table 1) show the highest plastid diversity in terms of number of detected haplotypes and parameters of molecular differentiation (Tables 2, 3). In contrast to *Q. suber*, in which the diversity was inflated by the occurrence of few divergent haplotypes linked to and likely captured from the 'Euro-Med' lineage of Sect. *Ilex* (H11-H12; overall haplotype diversity, h = 0.303), *Q. cerris* displayed a high number of *Cerris*-typical *trnH-psbA* variants, commonly shared with all the other species of the section (h = 0.538). This strikingly high haplotype richness, especially in the eastern part of this species' range (cf. Bagnoli et al., 2016) parallels the high morphological

plasticity of this oak (many different varieties and subspecies have been reported; for example, IOPI lists 30 *formae* and 17 varieties) and its ecological adaptability.

Quercus cerris has the largest range and broadest climatic envelope (from perhumid *Cfa, Cfb* via summer-dry warm temperate climates to *BSk*) and it is the only species of Sect. *Cerris* naturalized on the British Islands (*Cfb*) and cultivated all over continental Europe (mostly *Cfb*, sheltered *Dfb*). Indeed, establishment of a large range across the geologically and ecologically dynamic West Eurasian region might have provided many opportunities for diversification, isolation, drift, conservation of variants and eventual reticulation with sibling species. Likewise, *Q. cerris* also displayed a high nuclear (5S-IGS) diversity (Table 4, Fig. 3), with many instances of peculiar sequences (e.g., sample ce18, 22, 34, 50).

Quercus suber, instead, displayed the lowest number of unique 5S-IGS variants (like *Q. macrolepis*) with a low diversity (Table 4, 5), which might indicate ongoing genetic erosion (possibly due to the species domestication for cork, tannins, wood and fruits exploitation). Notwithstanding this, clones of the Iberian samples su07, su09, su29 with haplotype H11 (i.e. the Sect. *Ilex* 'Euro-Med' lineage) and H1 (the putative ancestral haplotype, see below) were highly divergent (Table 4, Fig. 3, File S4). In view of the high species-coherence detected in the other conspecific samples, this could either indicate introgression of *Q. suber* into *Q. ilex* or reflect ancient reticulation and retention of ancestral signatures.

In *Q. libani*, the high haplotype diversity co-incides with a moderate diversity at the 5S-IGS locus characterized by interesting variation among cloned sequences. Two individuals of this species, normally showing short 5S-IGS sequences (Denk & Grimm, 2010), exhibited the long variant (li01, the only specimen with haplotype H1, and li05, with haplotype H8, exclusive of *Q. libani*). These accessions could be introgressed specimens (e.g. with sympatric *Q. trojana* samples tj05, tj35, bearing haplotype H1 and highly similar 5S-IGS sequences; File S1, S4). Alternatively, they might represent an ancestral line of diversification within the section (the short variant also occurs in clones of two non-sympatric *Q. cerris* individuals). Together with *Q. brantii, Q. libani* is the easternmost oak among the western Eurasian *Cerris*, and it is extremely variable at the morphological and ecological level (occurring in climates ranging from *Csa* to *Dsb*). For instance, Djavanchir-Khoie (1967) described up to 12 intra-specific taxa within *Q. libani*, and introgression phenomena with other co-occurring oaks have been postulated (Menitsky, 2005; Khadivi-Khub et al., 2015). Likewise, in the nearby region of Iranian

Kurdistan, this oak showed three distinct gene pools based on nuclear microsatellites (Khadivi-Khub et al., 2015).

Based on the phylogenetic reconstructions, the 5S-IGS sequence diversity detected in *Q. trojana* outmatched the high levels displayed by *Q. cerris*. Sequences of both species were scattered all across the ML tree (Fig. 3), with a few clones close to the outgroup-inferred root of the tree (e.g. clones of samples tj24, tj45, ce29; tj39 at the root of clade 2). Samples with highly diverse clones were detected in both species (see Results), but individuals of *Q. trojana* can be found in two different clusters in the 5S-IGS network. Reflecting its more limited distribution and climatic niche (*Csa, Csb*), the haplotype diversity is substantially lower in *Q. trojana* s.l. (*Q. trojana* + *Q. euboica*) than in *Q. cerris* (Table 2, 3). This finding indicates that the two species retain exceptionally high intra- and inter-individual variability, possibly conserving ancestral variants lost in more homogenized and/or geographically restricted species of Sect. *Cerris*. This corresponds, at the plastid level, to the relative extension of their geographic (and ecological) ranges. Accordingly, the two endemic taxa *Q. afares* and *Q. castaneifolia*, genetically close to *Q. cerris* and *Q. trojana* but morphologically very distinct, appeared the least diverse (Table 4, 5). However, more data are needed for *Q. castaneifolia*, which was here represented by only two samples. The same holds for the two other taxa with narrow ranges in our dataset, *Q. euboica* and *Q. look*. Both are characterized by low levels of genetic diversty (Table 4, 5). However, despite the low number of individuals investigated, our results allow first taxonomic inferences in both cases.

Quercus euboica appears genetically isolated from *Q. trojana*, based on the number of unique 5S variants (Table 4) and the relative inter-taxa divergence (Table 5). This oak grows isolated from *Q. trojana* on the Greek island of Euboea and differs morphologically by its coriaceous leaf texture and the conspicuous white tomentum of the abaxial leaf surface that is made up of stellate trichomes (T. Denk, pers. observ.) In addition, *Q. euboica* is characterized by special edaphic conditions growing on serpentine rocks. All these data indicate that the Euboean oak should be better considered an independent species requiring special protection. Another (hairy) variant of *Q. trojana* has been locally described at the south-eastern margin of the species' range, in South-central Turkey (*Q. trojana* subsp. *yaltirikii;* Zielinski et al., 2006). Some samples collected in the nearby area (tj03, 04, 05, 16) showed 5S-IGS clones with a unique, highly divergent motif, and

grouped (mostly) in a specific sublade (Fig. 3, File S4). However, more (morpho-ecological) data are needed to implement the description of *Q. trojana* in this part of its range. Conversely, all the *Q. look* samples appeared distinct at the plastid and nuclear levels (Table 2–4). The 5S-IGS clones were strongly related to *Q. cerris* (shared sequence features and variants, see Results), and *Q. trojana* (included in the same clusters; File S4), whereas the plastid haplotypes were shared with the nearby *Q. cerris* and *Q. ithaburensis*. In addition, this species showed the lowest mean estimate of evolutionary divergence of the nuclear 5S IGS with *Q. castaneifolia* (Table 5). Although the precise taxonomic rank of this rare, enigmatic taxon cannot be established with certainty yet (a hybrid origin or a local diversification of an ancestral form of *Q. cerris* seem equally probable), the two previous assessments of this oak as synonym of *Q. ithaburensis* or *Q. ithaburensis* x *Q. libani* hybrid (Table 1) can be rejected. Additional investigations are required to evaluate if the morphology of *Q. look* justifies its exclusion from the genetically and morphologically variable *Q. cerris*.

Finally, a group with medium-high levels of plastid and nuclear diversity includes *Q. brantii, Q. ithaburensis,* and *Q. macrolepis* (Table 2, 4, 5; 'Aegilops oaks') with a range centred in the *Csa* climates of the central-eastern Mediterranean region, providing a low-land analogue to the situation in *Q. trojana-euboica-libani*. The Aegilops oaks are a highly specialized group, morphologically and ecologically well distinct from the other oaks in the *Cerris* section (Menitsky, 2005). The detected genetic diversity at the plastid and, especially, the nuclear markers (Table 5) clearly indicates the genetic isolation from the rest of the *Cerris* oaks and progressive inter-specific differentiation. Geographic, morphological and ecological differences are also evident in *Q. ithaburensis* and *Q. macrolepis* (Dufour-Dror & Ertas, 2002, 2004). On these grounds and considering the high inter-taxon 5S-IGS divergence supported by the different haplotypes (Tables 2–4), we suggest that the two forms should be treated as separate species (cf. Denk et al., 2017, appendix: http://dx.doi.org/10.1101/168146). Interestingly, *Q. brantii* appeared as the most diverse of the three taxa (Table 4, 5), and displayed some 5S-IGS variants shared with *Q. cerris* and *Q. suber* (File S3), which might indicate the presence of ancestral traits.

Hybrid detection within *Quercus* Section *Cerris*

Besides the deletion in *Q. libani*, some other sequence features, typical of other species (e.g., the SSR motif in *Q. brantii*, the deletion in *Q. suber*), confirmed the hybrid identity of a few samples included in our dataset (sample ml27, tj08 and tj33, supposed hybrids *Q. macrolepis* x *brantii*, *Q. trojana* x *Q. suber* and *Q. trojana* x *Q. libani*, respectively). The haplotypes of these samples (ml27: H6, exclusive of *Q. brantii*; tj08: H2, never found in *Q. suber*), also allowed identification of the maternal species. This finding confirms that these oaks can occasionally hybridize in sympatry, and evidence for such hybridization manifests in the nuclear genome (see also Fitzek et al., 2018).

Further instances of hybridization and/or introgression events could be inferred, based on common sequence features, the inter-specifically shared variants (see Results section, Table 4, Fig. 3, File S3), and the placement of the individuals in the AVG Neighbour-Net (Fig. 4), mostly involving Anatolian samples of *Q. cerris*, *Q. brantii*, *Q. libani*, *Q. macrolepis* and *Q. trojana*. However, in many cases the involved individuals were distant hundreds to a few thousands of kilometres each other. Based on their (relative) spatial proximity, introgressions could be suggested for samples *Q. brantii* (br02; C. Anatolia) and *Q. macrolepis* (e.g., ml20–22; W. and S. Anatolia) sharing sequence features, and South Anatolian *Q. libani* (li01) and *Q. trojana* (e.g., tj05, tj35) sharing sequence features and *trnH-psbA* haplotypes. Outside Anatolia, evidence of recent reticulation (shared variants) can be found between Israelian *Q. cerris* and *Q. look* (sample ce38, lk03) and between Balkan *Q. cerris* and *Q. suber* (sample ce43, Serbia; su37, Croatia). Introgression and past hybridization events between these species or their precursors is a possible explanation. At the same time, retention of ancestral traits cannot be discarded, as *Q. cerris* (5S-IGS, *trnH-psbA*) and *Q. trojana* (5S-IGS) cover most variability found in Sect. *Cerris* and are highly variable, especially in Anatolia.

In this context, the unresolved taxonomic status of *Q. crenata* can be discussed in view of the present results. Eight clones of samples cr04, cr05, cr06 (from regions where *Q. suber* is absent) showed an insertion shared with all species except the cork oak and were placed in the *Q. cerris*-dominated 'crown clade' 4 (Fig. 3), which is mostly composed of clones obtained from individuals of the 'occidental' lineage in Sect. *Cerris* (Figs 4, 5). At the same time, two other samples (from regions where the cork oak does occur) shared identical sequences with as many as 65 clones of *Q. suber* from across its entire range, and all the remaining clones (18 sequences, belonging to all six *Q. crenata* samples) clustered within the *Q. suber* clade, hence, the high

coherence of the *cerris-suber* lineage in the AVG and PBC networks. This finding can be interpreted as evidence of co-existing both (1) *Q. cerris* x *Q. suber* F1 hybrids and (2) introgressive forms into either *Q. cerris* (North East Italy/Balkans) or *Q. suber* (Italian peninsula), in partial agreement with Conte et al. (2007). However, all forms would look phenotypically quite similar (intermediacy of habitus, leaf and bark shape between *Q. suber* and *Q. cerris* is traditionally used as a macro-morphological diagnostic character of *Q. crenata*), which seems inconsistent with their presumable different genome composition. Clearly, the hybrid/introgressed phenotypes can be affected by several phenomena such as segregation, epistasis, heterosis, and maternal origin (Rieseberg & Ellestrad, 1993). At the same time, we note that the diagnostic traits used for *Q. crenata* occur in other oak species of the *Cerris* section (corky bark: *Q. afares*, *Q. variabilis*, various forms of *Q. cerris*; Menitsky, 2005; semi-evergreen habitus: *Q. trojana* and *Q. libani*; Yaltirik, 1984; crenate leaves: part of the morphological variation of *Q*, *cerris* and *Q. suber*). Also, *Q. cerris* is genetically not exclusive: plastid and nuclear signatures are shared with other species of Sect. *Cerris,* including geographically isolated, morphologically distinct, potential or commonly accepted species such as *Q. afares* (ce50)*, Q. castaneifolia*, *Q. euboica* (traditionally included in *Q. trojana*)*,* and *Q. look* (traditionally included in *Q. ithaburensis*), which are part of the 'occidental' lineage. Besides occasional hybridizations (sample cr05; Fig. 4), the alternative explanation is that *Q. crenata* represents a less-derived species possessing a limited gene pool within an autonomous evolutionary lineage including *Q. suber* (Fig. 5). Being closer to the common root, it retained imprints of common origin, possibly ancient reticulation, with the (proto-)*Q. cerris* ('occidental') lineage, representing a geographic-evolutionary gradient ('oriental' lineage → 'occidental' lineage → *crenata-suber* lineage). *Quercus crenata* may then just represent the remainder of the ancestral form from which *Q. suber* evolved rather than being the product of secondary contact between *Q. cerris* and *Q. suber.*

We also found no evidence to support the hybrid origin of *Q. afares* (*Q. suber* x *Q. canariensis*; the latter is a member of Sect. *Quercus*) as suggested by Mir et al. (2009) based on cpDNA-RFLP and allozymes (cf. Welter et al., 2012 and Mhamdi et al., 2013). 5S-IGS variants and plastid signatures of western Eurasian white oaks ('roburoid oaks' in Denk & Grimm, 2010; see also Simeone et al., 2016, fig. 1) are very distinct from *Cerris* types and should be detectable unless the F1 hybrids, with *Q. suber* as a maternal parent, only backcrossed with the local *Q.*

suber but not *Q. canariensis.* However, genetic exchange with local *Q. suber* can be excluded, since no *Q. suber*-typical 5S-IGS variants were found in *Q. afares*, nor *Q. afares* can be linked to the *crenata-suber* lineage (Figs 4, 5). Ongoing next-generation target sequencing of the 5S-IGS region (producing several 10,000 5S-IGS sequences per sample/individual) showed, so far, no evidence for a clone-sampling artefact in the studied individuals of *Q. afares.* The possibility of incomprehensive clone-sampling can thus be discarded. Analoguous to *Q. crenata,* the level of derivedness may explain earlier finds interpreted towards a hybrid origin: being much closer to the common ancestor of Sect. *Cerris* than *Q. suber*, this species may have retained (some) genetic imprints today found in members outside its section. This would explain also the association of *Q. afares* with two other, geographically very distant individuals of the 'oriental' lineage (ce50, li05, the only *Q. libani* without the 'short' *libani* 5S-IGS variants, but showing variants similar to Anatolian *Q. trojana*).

Aside from putative hybrid species and swarms (see also the ambiguous placement of sample it03 the 5S-IGS network), our data demonstrates a general permeability of species boundaries in members of Sect. *Cerris*, allowing occasional crosses. Indeed, our results demonstrate that *Q. trojana* and *Q. libani*, *Q. brantii* and *Q. macrolepis*, *Q. cerris* and *Q. suber*, and *Q. trojana* and *Q. suber* are interfertile and hybridize in the wild. Further investigations are needed to distinguish between ancient hybridization with subsequent incomplete lineage sorting and retention of ancestral traits, to clarify the status of several other samples that may represent both phenomena based on shared 5S-IGS variants, the occurrence of unique sequence features, length of terminal branches and odd-placing in the phylogenetic reconstructions. Adequately addressing these issues would be of great relevance to identify relict populations and/or past contact/hybrid zones, to assess the hybridization ability of species growing in sympatry, and further define the evolutionary history of the *Cerris* oaks.

Taxonomic framework of *Quercus* section *Cerris* in western Eurasia

From the clusters identified by the 5S-IGS network based on the average inter-individual clone (Fig. 4) and PBC-transformed distances (Fig. 5), four major groups can be identified representing distinct evolutionary lineages and used as a framework. Figure 6 shows a scheme, a cactus-type branching silhouette (Podani, 2017; Morrison 2018), based on the 5S-IGS and *trnH-psbA* differentiation patterns, and with respect to the plastid tree provided in Simeone et al. (2016).

The first, most western lineage includes *Q. suber* and *Q. crenata*. Sample cr05 likely represents a F1 hybrid with *Q. cerris*. The same holds for tj08, a *Q. trojana* x *Q. suber* cultivated hybrid in the Botanical Garden of Naples. A second lineage, tentatively termed the 'occidentalis' lineage includes the widespread *Q. cerris* and the geographically restricted *Q. castaneifolia, Q. euboica* and *Q. look*. The third lineage collects the Eastern Mediterranean 'Vallonea' oaks *Q. brantii*, *Q. ithaburensis,* and *Q. macrolepis* with three potential outliers: br02, br03 and it03, placed apart because of the admixture of lineage 2-type clones (possible recent or ancient hybrids). The last, least coherent group, the 'oriental' lineage, includes *Q. afares*, *Q. libani* and *Q. trojana*, with the only exception of sample li01, another possible recent or ancient hybrid. Aside from supposed hybrids and introgressed individuals, these groups almost perfectly match previous taxonomic observations (Sect. *Heterobalanus* Subsect. *Suber* + Sect. *Cerris* Subsect. *Cerris* and *Aegilops*; Menitsky, 2005; Sect. *Suber* + Sect. *Eucerris*, *Aegilops* and *Erythrobalanus*; Schwarz, 1936), with the only exceptions of *Q. afares*, accomodated by both authors within the *Q. cerris*-*Q. castaneifolia* group, and *Q. crenata* and *Q. look* that were not included in previous monographs. *Quercus trojana* was the only species scattered over two clusters (cluster 1 and 2, Figs 4, 5; see also Denk & Grimm, 2010), thus bridging between the 'oriental' and 'occidental' lineage. No geographic, haplotypic or subspecific relationships could explain this subdivision; it might therefore indicate the occurrence of two different, geographically overlapping but genetically isolated lineages within the species, possibly differentiated in the past (retained ancient polymorphism), especially considering the proximal positions of the sequences of samples tj24, 39 and 45 in the ML tree. Both *Q. trojana* (s.str.) sublineages occur in Italy and might correspond to the two main nuclear gene pools identified by Carabeo et al. (2017). Indeed, more ecological and molecular data are required to interpret this finding biologically.

Overall, the complex genetic differentiation patterns can only be explained by longer ongoing free genetic exchange or more recent common origin of the 'oriental' and 'occidental' lineages than in case of the other two lineages within western Eurasian *Cerris*: the corkish oaks (*Q. crenata* + *Q. suber*) and the 'Vallonea' (or Aegilops) oaks (*Q. brantii*, *Q. ithaburensis,* and *Q. macrolepis*). *Quercus euboica* possibly originated by geographical isolation from a proto-*trojana* still close to the proto-*cerris* (Fig. 6). Interestingly, all the *Q. euboica* samples occurred in the *Q. cerris*-dominated 'crown' clade 4 (Fig. 3), hence, are part of the 'occidentalis' lineage. Likewise, the microspecies *Q. afares, Q. castaneifolia,* and *Q. look* as well as the eastern replacement of *Q.*

669 *trojana, Q. libani,* were isolated from the master *cerris-trojana* genepool(s). It is impossible to
670 provide absolute dates for the final isolation events. Comparison with intra- and inter-species 5S-
671 IGS divergence in the other two main lineages of western Eurasian oaks ('ilicoid' oaks of Sect.
672 *Ilex*, 5S-IGS is species-diagnostic; 'roburoid' oaks of sections *Quercus* and *Ponticae*, 5S-IGS is
673 largely undiagnostic; Denk & Grimm, 2010) indicates that the original split possibly predates
674 diversification in the 'roburoid' oaks. The final establishment of species in western Eurasian
675 members of Sect. *Cerris* is however as young as in the 'roburoid' oaks, may be ongoing (*Q.*
676 *trojana*), and younger than the main split within the 'ilicoid' oaks, i.e. of *Q. ilex* from *Q. aucheri*
677 (+ *Q. coccifera*).
678
679 **Phylogeography of *Quercus* Section *Cerris* in western Eurasia**
680 The primitive (L1) and derived (L2) haplotype groups of 'Cerris-Ilex' lineage, and two
681 haplotypes representing the 'Euro-Med' lineage characteristic for western populations of Sect.
682 *Ilex* (Simeone et al., 2016; Vitelli et al., 2017), describe the main evolutionary trajectories of the
683 western Eurasian lineage of Sect. *Cerris* and their contact with already established lineages of
684 Sect. *Ilex*. According to coalescent theory, the most frequent and widespread haplotypes, i.e. H1
685 and H2, are likely ancestral (Posada & Crandall, 2001). This is confirmed by our haplotype
686 network (Fig. 1). The close relationship between haplotypes H1 and H2 – found all across
687 western Eurasia (Fig. 2b) and in all taxa except the south-eastern species group, *Q. brantii*, *Q.*
688 *look,* and *Q. ithaburensis* – and *Q. phylliraeoides* (Fig. 5; cf. Simeone et al., 2016; Fig. 1), the
689 only species of section *Ilex* extending into Japan – points towards a north-eastern Asian origin of
690 Sect. *Cerris* and a westward migration of a large population into the Mediterranean region. The
691 revised (see Introduction) fossil record of *Cerris* in (North-)East Asia (starting from early
692 Oligocene) predates earliest records in western Eurasia (Oligocene/Miocene boundary) by ca. 10
693 Ma, thus rejecting the hypothesis that Sect. *Cerris* evolved from the western stock of Sect. *Ilex*
694 populations with 'WAHEA' haplotypes (Denk & Grimm, 2010; Simeone et al., 2016). The
695 effective population size of the early west-migrating *Cerris* must have been (very) large in
696 contrast to their East Asian siblings. The East Asian species of Sect. *Cerris* are more
697 heterogenous (Chen et al., 2012; Zhang et al., 2015), and differ much more profoundly from *Q.*
698 *phylliraeoides* (the Japanese *Ilex* oak), but also from the 'WAHEA' haplotypes of Sect. *Ilex* and
699 *Q. baroni* (Fig. 1), considered early diverged plastid lineages of Subgenus *Cerris* ('Old World'

or mid-latitude clade; the earliest diverged plastid lineage being the western Mediterraenan 'Euro-Med' type found in *Q. ilex*; Fig. 5; Simeone et al., 2016).

Once established in the Mediterranean region (H1, H2; Fig. 2b), local bottlenecks may have contributed to increased genetic drift in the plastome in the eastern part of the range. A likely trigger are the complex orogenies shaping modern-day Turkey and the Levant, areas with an increased haplotype diversity including the most derived 'Cerris-Ilex' haplotypes (Figs 1–2). This, and the general west-east differentiation pattern (see also Figs 4 and 5), parallels the situation in Sect. *Ilex*, *Q. coccifera* in particular (Vitelli et al., 2017). A notable difference to Sect. *Ilex* is the lack of plastid structuring (and diversity) in the central and western Mediterranean region, indicating a rather recent, singular colonization by the master population, clearly not affected by Oligocene micro-plate tectonics as suggested for *Q. suber* by Magri et al. (2007).

The derived L2 'Cerris-Ilex' haplogroup (H5–H10) starts in Anatolia and extends further east (Iran) and south (Levant). In addition to isolation during range establishment, specialization to drier climates (e.g. summer-dry Mediterranean climates: *Csa, Csb, Dsb*) can be considered as trigger for increased genetic drift, possibly linked to speciation. The Aegilops oaks, *Q. brantii, Q. ithaburensis,* and *Q. macrolepis*, a well-circumscribed group based on 5S-IGS differentiation (Figs 4, 5) and morphology, are unique by showing only derived 'Cerris-Ilex' haplotypes.

A remarkable exception are two Italian *Q. cerris* individuals showing the derived haplotypes H9/H10, which occur in locations more than 2000 km apart from other individuals of this Levantine haplotype sublineage. H9/H10 derive from types found in Anatolia and eastwards (Figs 1, 2). Long-distance seed dispersal is highly unlikely. The main animal vector for propagation of oaks are the jaybirds, which are sedentary birds, with a short evasion range (< 50 km; Haffer & Bauer, 1993). Man-mediated dispersal (in historic times) could be a likely explanation, although we note that haplotypes shared by disjunct central Mediterranean and the Anatolian regions were also found in *Q. ilex*, and possibly reflect the remnants of a pre-Quaternary continuous range.

In this context, the genetic diversity detected in the Italian *Q. trojana* populations (both at the nuclear and at the plastid level) and the very limited, amphi-Adriatic distribution of haplotype H2 in *Q. macrolepis* (Italy, Albania; Fig. 2, File S1) likely confirm that these oaks are native in Italy. Similar close intra-specific phylogeographic relationships have been detected in other plant

731 species on both sides of the Adriatic Sea (Musacchio et al., 2006; Hilpold et al., 2014), including
732 oaks (Lumaret et al., 2002; Fineschi et al., 2002; de Heredia et al., 2007; Bagnoli et al., 2016). In
733 this case also, the Apulian populations of *Q. trojana* and *Q. macrolepis* can be interpreted as the
734 remnants of a once continuous ancestral range (Simeone et al., 2016), or witness a colonization
735 wave that was likely favoured by land connections between the Balkans and southeastern Italy
736 during the Messinian salinity crisis and (or) the Pleistocene glaciations (Nieto Feliner, 2014).
737
738 **Conclusion**
739
740 The present study is the first to include all putative species of *Quercus* Section *Cerris* in western
741 Eurasia. Our investigation is based on a dense intra-specific and geographic sampling and makes
742 use of DNA sequence variation of the two most divergent nuclear and plastid regions known for
743 oaks. The obtained results confirm and emend species relationships and their genetic coherence.
744 An upgraded subsectional allocation of the western Eurasian *Cerris* oak species is achieved, with
745 the identification of four major lineages, corresponding to subsectional groups that would need to
746 be formalized. The recognition of a number of infraspecific taxa as objective species (i.e., *Q.*
747 *macrolepis* and *Q. euboica*) is supported, and the correct taxonomic relationships of *Q. look* are
748 newly defined. We finally acknowledge the occurrence of occasional F1 hybrids, possible
749 intrograded individuals and several potential outlier individuals all across the studied range but
750 question the hybrid origin of *Q. afares* and *Q. crenata*. The fossil record corroborates major
751 inferences about the origin and diversification of the section. These data are important additions
752 to recent studies of other *Quercus* sections (see Introduction) improving our knowledge on oak
753 biodiversity and evolution.
754 Characterizing nuclear and plastid differentiation across all species and the entire range can only
755 be the first step. Figure 5 summarizes our results, but also highlights phenomena deserving
756 further investigation. Primarily, 5S-IGS data need to be compiled for (East) Asian members of
757 sections *Cerris* and *Ilex.* A future focus should be on all Hindukush to western Himalayan
758 species and the Japanese *Q. phylliraeoides,* the north-easternmost member of Sect. *Ilex*, which
759 has a plastid very similar to the western Eurasian members of Sect. *Cerris* but not to the
760 geographically much closer East Asian species of Sect. *Cerris*. The entire fossil record of
761 sections *Cerris* and *Ilex* should then be recruited to infer age estimates, following the recent

762 example of beech trees (Renner et al., 2016). Another open question is where to root the nuclear
763 tree (hence, the polytomy in Fig. 6): our incomprehensive outgroup places the root within the
764 *crenata-suber* portion of the 5S-IGS ML tree, which would mean that the 'corkish' oaks
765 represent the first diverging lineage. This rooting hypothesis does not fit well the structure of the
766 PBC network and would collide with the plastid and fossil evidence favouring a north-eastern
767 origin of the section. A stepwise East to West invasion of Sect. *Cerris* into the Mediterranean
768 region is also supported by higher species and plastid diversity in the East Mediterranean. A
769 hypothesis worth testing for bridging this gap would be that the westernmost ancestral
770 populations of the cerroid oaks, carrying the commonly shared haplotype, went through a
771 relatively recent bottleneck resulting in unique and distinct 5S-IGS variants, the latter attracted to
772 any possible (distant) outgroup when inferring a tree (ingroup-outgroup branching artefact; cf.
773 position of outgroups in Fig. 4). Recent, because the time was not sufficient to differentiate the
774 plastid pool of *crenata-suber* in contrast to e.g. the central-eastern Mediterranean Aegilops oaks,
775 covering a similar geographic and climatic range. Last, but not least, *Q. cerris* should be
776 investigated in detail across its entire range using a combination of morphometric and high-
777 resolution genetic analysis with respect to sympatric species of sect. *Cerris* and the isolated
778 endemics. This will allow testing whether *Q. cerris* is a primal genetic and ecological resource of
779 the section in western Eurasia and carrier of ancestral signals.

780 **References**

781

782 Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search
783 tool. J. Mol. Biol. 215:403-410.
784 Bagnoli F, Tsuda Y, Fineschi S, Bruschi P, Magri D, Zehlev P, Paule L, Simeone MC, González-
785 Martínez SC, Vendramin GG (2016) Combining molecular and fossil data to infer demographic
786 history of *Quercus cerris*: insights on European eastern glacial refugia. J. Biogeogr. 43: 679–
787 690.
788 Barak RS, Hipp AL,Cavender-Bares J, Pearse WD, Hotchkiss SC, Lynch EA, Callaway JC,
789 Calcote R, Larkin DJ (2016) Taking the long view: Integrating recorded, archeological,
790 paleoecological, and evolutionary data into ecological restoration. Int. J. Plant Sci. 177:90–102.
791 2016.
792 Belahbib N, Pemonge MH, Ouassou A, Sbay H, Kremer A, Petit RJ (2001) Frequent
793 cytoplasmic exchanges between oak species that are not closely related: *Quercus suber* and *Q.*
794 *ilex* in Morocco. Mol. Ecol. 10:2003–2012.
795 Bellarosa R, Simeone MC, Papini A, Schirone B (2005) Utility of ITS sequence data for
796 phylogenetic reconstruction of Italian *Quercus* spp. Molecular Phylogenetics and Evolution 34:
797 355–370.
798 Browicz K, Zieliński J (1982) Chorology of trees and shrubs in South-West Asia and adjacent
799 regions, Vol. 1. Warsaw: Polish Scientific Publishers
800 Bryant D, Moulton V (2004) Neighbor-Net: an agglomerative method for the construction of
801 phylogenetic networks. Mol Biol Evol 21: 255–265
802 Burgarella C, Lorenzo Z, Jabbour-Zahab R, Lumaret R, Guichoux E, Petit R, Soto A, Gil L
803 (2009) Detection of hybrids in nature: application to oaks (*Quercus suber* and *Q. ilex*). Heredity
804 102: 442-452
805 Burger WC (1975) The species concept in *Quercus*. Taxon 24: 45–50.
806 Camus A (1936–54) Les chênes. Monographie du genre *Quercus* et monographie du genre
807 *Lithocarpus*. Encyclopédie Economique de Sylviculture, Vol. VI, VII, VIII. Paris: Lechevalier.
808 Carabeo M, Simeone MC, Cherubini M, Mattia C, Chiocchini F, Bertini L, Caruso C, La Mantia
809 T, Villani F, Mattioni C (2017) Estimating the genetic diversity and structure of *Quercus trojana*

Webb populations in Italy by SSRs: implications for management and conservation. Can J For Res 47: 331–339.

Cavender-Bares J, Gonzalez-Rodriguez A, Eaton DAR, Hipp AAL, Beulke A, Manos PS (2015) Phylogeny and biogeography of the American live oaks (*Quercus* subsection *Virentes*): A genomic and population genetics approach. Mol Ecol 24: 3668–3687

Chen DM, Zhang XX, Kang HZ, Sun X, Yin S, Du HM. et al. (2012) Phylogeography of *Quercus variabilis* based on chloroplast DNA sequence in East Asia: multiple glacial refugia and Mainland-migrated island populations. PLoS ONE 7:e47268.

Chen J, Zeng Y-F, Liao W-J, Yan P-C, Zhang J-G (2017) A novel set of single-copy nuclear gene markers in white oak and implications for species delimitation. Tree Genetics and Genomes 13: 50

Conte L, Cotti C, Cristofolini G (2007) Molecular evidence for hybrid origin of *Quercus crenata* Lam. (Fagaceae) from *Q. cerris* L. and *Q. suber* L. Plant Biosystems: 141:181–193.

Deng M, Jiang X-L, Hipp AL, Manos PS, Hahn M (2018) Phylogeny and biogeography of East Asian evergreen oaks (*Quercus* section *Cyclobalanopsis*; Fagaceae): Insights into the Cenozoic history of evergreen broad-leaved forests in subtropical Asia, Molecular Phylogenetics and Evolution, 119, 170-181.

Denk T, Grimm GW, Manos PS, Deng M, Hipp AL (2017) An Updated Infrageneric Classification of the Oaks: Review of Previous Taxonomic Schemes and Synthesis of Evolutionary Patterns. In: Gil-Pelegrín E, Peguero-Pina J, Sancho-Knapik D (eds) Oaks Physiological Ecology. Exploring the Functional Diversity of Genus *Quercus* L.. Tree Physiology, vol 7. Springer, Cham

Denk T, Grimm GW (2010) The oaks of western Eurasia: traditional classifications and evidence from two nuclear markers. Taxon 59: 351-366

Djavanchir-Khoie K (1967) Les chenes de l'Iran. Ph.D. thesis, Univ. Montpellier, 221 pp

Excoffier L, Foll M, Petit RJ (2009) Genetic Consequences of Range Expansions. Ann. Rev. Ecol. Evol. Syst. 40: 481-501

Dufour-Dror JM, Ertas A (2002) Cupule and acorn basic morphological differences between Quercus *ithaburensis* Decne. subsp. *ithaburensis* and *Quercus ithaburensis* subsp. *macrolepis* (Kotschy) Hedge & Yalt. Acta Botanica Malacitana 27: 237–242.

840 Dufour-Dror JM, A Ertas (2004) Bioclimatic perspectives in the distribution of *Quercus*
841 *ithaburensis* Decne. subspecies in Turkey and in the Levant. Journal of Biogeography 31: 461-
842 474.
843 Fang J, Wang Z, Tang Z (2009) Atlas of Woody Plants in China. Volumes 1 to 3 and index.
844 Beijing: Higher Education Press.
845 Fineschi S, Taurchini D, Grossoni P, Petit RJ, and Vendramin GG (2002) Chloroplast DNA
846 variation of white oaks in Italy. For. Ecol. Manag. 156:103–114.
847 Fitzek E, Delcamp A, Guichoux E, Hahn M, Lobdell M, Hipp AL (2018) A nuclear DNA
848 barcode for eastern North American oaks and application to a study of hybridization in an
849 Arboretum setting. Ecology and Evolution 00:1–15
850 Forest F, Savolainen V, Chase MW, Lupia R, Bruneau A, Crane PR (2005) Teasing apart
851 molecular- versus fossil-based error estimates when dating phylogenetic trees: a case study in the
852 birch family (Betulaceae). Systematic Botany 30:118-133.
853 Göker M, Grimm GW (2008) General functions to transform associate data to host data, and
854 their use in phylogenetic inference from sequences with intra-individual variability. Bmc Evol.
855 Biol. 8: 86
856 Govaerts R, Frodin DG (1998) World checklist and bibliography of Fagales (Betulaceae,
857 Corylaceae, Fagaceae and Ticodendraceae). Royal Botanic Gardens, Kew
858 Grimm GW, Denk T (2010) The reticulate origin of modern plane trees (*Platanus*, Platanaceae) -
859 a nuclear marker puzzle. Taxon 59:134-147.
860 Grímsson F, Grimm GW, Zetter R, Denk T (2016) Cretaceous and Paleogene Fagaceae from
861 North America and Greenland: evidence for a Late Cretaceous split between Fagus and the
862 remaining Fagaceae. Acta Palaeobot 56:247–305
863 Hipp AL, Eaton DAR, Cavender-Bares J, Fitzek E, Nipper R, Manos PS (2014) A framework
864 phylogeny of the American oak clade based on sequenced RAD data. Plos One 9: e93975
865 Hipp AL, Manos PS, Gonzalez-Rodriguez A, Hahn M, Kaproth M, McVay JD, Valencia-A S,
866 Cavender-Bares J (2018) Sympatric parallel diversification of major oak clades in the Americas
867 and the origins of Mexican oak diversity. New Phytologist 217: 439-452
868 Hubert F, Grimm GW, Jousselin E, Berry V, Franc A, Kremer A (2014) Multiple nuclear genes
869 stabilize the phylogenetic backbone of the genus *Quercus*. Systematics and Biodiversity 12:405–
870 423.

871 Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Mol
872 Biol Evol 23: 254–267
873 Khadivi-Khub A, Shabanian N, Alikhani L, Rahmani M-S (2015) Genotypic analysis and
874 population structure of Lebanon oak (*Quercus libani* G. Olivier) with molecular markers. Tree
875 Genet. Genomes 11:102
876 Kottek M, Grieser J, Beck C, Rudolf B, Rubel F (2006) World map of the Köppen-Geiger
877 climate classification updated. Meteorologische Zeitschrift 15: 259-263
878 Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis
879 version 7.0 for bigger datasets. Mol. Biol. Evol. 33:1870-1874
880 Lehtonen S, Myllys L (2008) Cladistic analysis of *Echinodorus* (Alismataceae): simultaneous
881 analysis of molecular and morphological data. Cladistics 24:218-239.
882 Librado P, Rozas J (2009) DnaSP v5: A software for comprehensive analysis of DNA
883 polymorphism data. Bioinformatics 25: 1451–1452
884 López de Heredia U, Jiménez P, Collada C, Simeone MC, Bellarosa R, Schirone B, Cervera MT,
885 Gil L (2007) Multi-marker phylogeny of three evergreen oaks reveals vicariant patterns in the
886 Western Mediterranean. Taxon 56: 1209-1209
887 Lumaret R. and Jabbour-Zahab R. (2009) Ancient and current gene flow between two distanly
888 related Mediterranean oak species, *Quercus suber* and *Q. ilex*. Annals of Botany 104: 725-736.
889 Lumaret R, Mir C, Michaud H, Raynal V (2002) Phylogeographic variation of chloroplast DNA
890 in holm oak (*Q. ilex* L.). Mol. Ecol. 11: 2327–2336.
891 Magri D, Fineschi S, Bellarosa R, Buonamici A, Sebastiani F, Schirone B, Simeone MC,
892 Vendramin GG (2007) The distribution of *Quercus suber* choloroplast haplotypes matches the
893 palaeogeographic history of the western Mediterranean. Molecular Ecology 16, 5259-5266
894 Manos PS, Zhou ZK, Cannon CH (2001) Systematics of Fagaceae: phylogenetic tests of
895 reproductive trait evolution. International Journal of Plant Science 162: 1361–1379.
896 McVay JD, Hipp AL, Manos PS (2017) A genetic legacy of introgression confounds phylogeny
897 and biogeography in oaks. Proceedings of the Royal Society B 284:20170300
898 Menitsky YL (2005) Oaks of Asia. Science Publishers, Enflied, New Hampshire, USA
899 Mhamdi S, Brendel O, Montpied P, Ghouil-Amimi H, Hasnaoui I, Dreyer E (2013) Leaf
900 morphology displays no detectable spatial organisation in the relict *Quercus afares* Pomel

compared to the co-occurring parental species *Q. canariensis* Willd. and *Q. suber* L. Ann. For. Sci 70:675-684.

Mir C, Toumi L, Jarne P, Sarda V, Di Giusto F, Lumaret R (2006) Endemic North African *Quercus afares* Pomel originates from hybridisation between two genetically very distant oak species (*Q. suber* L. and *Q. canariensis* Willd.): evidence from nuclear and cytoplasmic markers. Heredity 96:175–184.

Morrison D (2018) Tree metaphors and mathematical trees. Genealogical World of Phylogenetic Networks; http://phylonetworks.blogspot.com/2018/02/tree-metaphors-and-mathematical-trees.html

Muir G, Fleming CC, Schlotterer C (2001)) Tree divergent rDNA clusters predate the species divergence in *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. Mol. Biol. Evol. 18: 112–119.

Musacchio A, Pellegrino G, Cafasso D, Widmer A, Cozzolino S (2006) A unique *A. palustris* lineage across the Otranto strait: botanical evidence for a past land-bridge? Plant Syst. Evol. 262: 103–111.

Nieto Feliner G (2014) Patterns and processes in plant phylogeography in the Mediterranean Basin. A review. Perspect. Plant. Ecol. Evol. Syst. 16: 265–278.

Oh S-H Manos PS (2008) Molecular phylogenetics and cupule evolution in Fagaceae as inferred from nuclear CRABS CLAW sequences. Taxon 57: 434–451.

Pattengale ND, Masoud A, Bininda-Emonds ORP, Moret BME, Stamatakis A. 2009. How many bootstrap replicates are necessary? In: Batzoglou S, ed. *RECOMB 2009*. Berlin, Heidelberg: Springer-Verlag, p. 184–200.

Pautasso M (2009) Geographical genetics and conservation of forest tree. Perspect. Plant. Ecol. Evol. Syst. 11: 157–189.

Peel MC, Finlayson BL, McMahon TA (2007) Updated world map of the Köppen-Geiger climate classification. Hydrology and Earth System Sciences 11: 1633-1644

Petit RJ, Bodénès C, Ducousso A, Roussel G, Kremer A (2004) Hybridization as a mechanism of invasion in oaks. New Phytologist 161: 151-164

Pham KK, Hipp AL, Manos PS, Cronn RC (2017) A time and a place for everything: phylogenetic history and geography as joint predictors of oak plastome phylogeny. Genome 60: 720-732

Podani J (2017) Different from trees, more than metaphors: branching silhouettes — corals, cacti, and the oaks. Systematic Biology 66: 737-753.

Posada D, Crandall KA (2001) Intra-specific gene genealogies: trees grafting into network. Trends in Ecology and Evolution 16: 37–45

Renner SS, Grimm GW, Kapli P, Denk T (2016) Species relationships and divergence times in beeches: New insights from the inclusion of 53 young and old fossils in a birth-death clock model. Philosophical Transactions of the Royal Society B 371:20150135

Rieseberg LH, Ellstrand NC (1993) What can molecular and morphological markers tell us about plant hybridization? Critical Reviews in Plant Sciences 12: 213-241.

Rubel F, Brugger K, Haslinger K, Auer I. 2016. The climate of the European Alps: Shift of very high resolution Köppen-Geiger climate zones 1800–2100. *Meteorologische Zeitschrift* 26:115-125

Schroeder G-F (1998) Lehrbuch der Pflanzengeographie. Wiesbaden: Quelle & Meyer.

Schwarz O (1936-39) Monographie der Eichen Europas und des Mittelmeergebietes. Feddes Repertorium regni vegetabilis. Berlin-Dahlem: Sonderbeiheft D.

Simeone MC, Grimm GW, Papini A, Vessella F, Cardoni S, Tordoni E, Piredda R, Franc A, Denk T (2016), Plastome data reveal multiple geographic origins of *Quercus* Group *Ilex*. PeerJ 4:e1897.

Simeone MC, Piredda R, Papini A, Vessella F, Schirone B (2013) Application of plastid and nuclear markers to DNA barcoding of Euro–Mediterranean oaks (*Quercus*, Fagaceae): problems, prospects and phylogenetic implications. Botanical Journal of the Linnean Society 172, 478-499

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312-3.

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673-4680

Van Valen L (1976) Ecological species, multispecies, and oaks. Taxon 25: 233–239.

Vitelli M, Vessella F, Cardoni S, Pollegioni P, Denk T, Grimm GW, Simeone MC (2017) Phylogeographic structuring of plastome diversity in Mediterranean oaks (*Quercus* Group *Ilex*, Fagaceae). Tree Genetics and Genomes 13:3.

962 Volkov RA, Zanke C, Panchuk I, Hemleben V. 2001. Molecular evolution of 5S rDNA of
963 *Solanum* species (sect. *Petota*): application for molecular phylogeny and breeding. *Theoretical*
964 *and Applied Genetics* 103:1273-1282.
965 Walter H, Breckle S-W (1983–1991) Ökologie der Erde (4 volumes). Stuttgart: Eugen Ulmer
966 Verlag.
967 Welter S, Bracho-Nuñez A, Mir C, Zimmer I, Kesselmeier J, Lumaret R, Schnitzler JP, Staudt M
968 (2012) The diversification of terpene emissions in Mediterranean oaks: lessons from a study of
969 Quercus suber, Quercus canariensis and its hybrid Quercus afares. Tree Physiol 32:1082–1091.
970 Yaltirik F. 1984, Türkiye Meşeleri Teşhis Klavuzu, İstanbul.
971 Yang J, Vázquez L, Chen X, Li H, Zhang H, Liu Z and Zhao G (2017) Development of
972 Chloroplast and Nuclear DNA Markers for Chinese Oaks (*Quercus* Subgenus *Quercus*) and
973 Assessment of Their Utility as DNA Barcodes. Front. Plant Sci. 8:816.
974 Zielinski J, Petrova A, Tomaszewski D (2006) *Quercus trojana* subsp. *yaltirikii* (Fagaceae), a
975 new subspecies from southern Turkey. Willdenowia 36, 845-849
976 Zhang X W, Li Y, Liu CY, Xia T, Zhang Q, Fang YM (2015). Phylogeography of the temperate
977 tree species *Quercus acutissima* in China: Inferences from chloroplast DNA variations.
978 Biochem. Syst. Ecol. 63, 190–197.

Table 1(on next page)

Species and taxa included in *Quercus* Section *Cerris*.

Nomenclature followed Govaerts & Frodin (1998); species investigated in the present study are bolded. Taxonomic remarks and species distributions according to *Govaerts & Frodin (1998) and **Menitsky (2005)

1

Taxon	Taxonomic remarks	Distribution
***Q. afares* Pomel**		Endemic, Algerian and Tunisian Tell Atlas
***Q. brantii* Lindl.**		S and SE Anatolia to Iran, Lebanon
***Q. castaneifolia* C.A. Mey**		Endemic; SE Caspian Sea, Azerbaijan to Iran
***Q. cerris* L.**		E and C Mediterranean, Balkans
***Q. crenata* Lam.**	* Poorly known	Endemic, Italian peninsula
***Q. libani* Oliv.**		SE Anatolia to Iran
***Q. look* Kotschy**	* Synonym of *Q. ithaburensis* ssp. *ithaburensis;* ** hybrid *Q. ithaburensis* x *Q. libani*	Endemic, Lebanon to Anti-Lebanon mountain range
***Q. ithaburensis* Decne.**	** Including ssp. *macrolepis* (Kotschy), distributed in the European part of the range, and ssp. *ithaburensis* (Decaisne), in the Middle East	E Mediterranean, SE Italy to Palestine
***Q. trojana* Webb**	* Including ssp. *trojana* and ssp. *euboica* (Papaioann.) K.I.Chr., endemic of Euboea (Greece)	Anatolia, Aegean to SE Italy
***Q. suber* L.**		C and W Mediterranean
Q. acutissima Carruth.		E and SE Asia
Q. chenii Nakai		E Asia
Q. variabilis Blume		E and SE Asia, Japan

2

Table 2 (on next page)

Diversity values of the *trnH-psbA* IGS in the investigated dataset.

N: number of sequences; L: Aligned length (bp) with the inversion deleted; *p*: uncorrected *p*-distance range (STD); H: Number of identified haplotypes (gaps included); h: Haplotype diversity; Hid: haplotype code; S: Number of polymorphic sites (gaps included); PICs: Number of Parsimony Informative Characters; * including ssp. *euboica*; ** including ssp. *macrolepis;* † GenBank haplotype accessions: KT152191-KT152200, JF753573-JF753583, KM210647, HE585136; ‡: no haplotype shared with the West Eurasian dataset, one haplotype shared between the two East Asian species.

1
2

Dataset	N	L	*p*	H	h	Hid	S	PICs
West Eurasian species	207	503	0,000 – 0,008 (±0,004)	12	0,515	H1-H12	6 (27)	6
Q. afares	7	491	0,000	1	0,000	H1	0	0
Q. brantii	7	487	0,000	2	0,476	H5, H6	0 (1)	0
Q. cerris	52	493	0,000 – 0,002	8	0,538	H1-H3, H5-H7, H9, H10	1 (9)	1
Q. castaneifolia	2	491	0,000	1	0,000	H1	0	0
Q. crenata	6	491	0,000	1	0,000	H1	0	0
*Q. trojana**	45	493	0,000 – 0,002	3	0,369	H1, H2, H4	1 (3)	1
*Q. ithaburensis***	33	493	0,000 – 0,002	5	0,655	H1, H2, H4, H6, H9	1 (8)	1
Q. look	3	488	0,000	2	0,667	H9, H10	0 (1)	0
Q. suber	47	501	0,000 – 0,006	3	0,303	H1, H11, H12	3 (18)	3
Q. libani	5	493	0,000 – 0,002	3	0,700	H1, H3, H8	1 (8)	0
Q. acutissima†	401	564	0,000 – 0,004	10	n.d.	/‡	4 (79)	0
Q. variabilis†	528	594	0,000 – 0,004	11	n.d.	/‡	2 (99)	0

3
4

Table 3(on next page)

Heatmap with the mean estimates of evolutionary divergence of the *trnH-psbA* IGS over sequence pairs within and between the investigated taxa.

† GenBank haplotype accessions as in Table 2; * including ssp. *euboica*; ** including ssp. *macrolepis*; standard error estimate are shown above the diagonal.

1

Dataset	Intra-	Interspecies divergence											
Q. afares	0		0	0,0001	0	0	0,0001	0,0001	0,0004	0,0009	0,0006	0,0004	0,0002
Q. brantii	0	0		0,0001	0	0	0,0001	0,0002	0,0004	0,0015	0,0006	0,0004	0,0002
Q. cerris	0,0003	0,0002	0,0002		0,0001	0,0001	0,0001	0,0002	0,0004	0,0008	0,0006	0,0004	0,0002
Q, castaneifolia	0	0	0	0,0002		0	0,0001	0,0001	0,0004	0,0009	0,0006	0,0004	0,0002
Q. crenata	0	0	0	0,0002	0		0,0001	0,0001	0,0004	0,0009	0,0006	0,0004	0,0002
*Q. trojana**	0,0001	0,0001	0,0001	0,0003	0,0001	0,0001		0,0001	0,0004	0,001	0,0006	0,0004	0,0002
*Q. ithaburensis***	0,0003	0,0001	0,0003	0,0003	0,0001	0,0001	0,0002		0,0005	0,001	0,0006	0,0004	0,0002
Q. libani	0,0008	0,0004	0,0004	0,0006	0,0004	0,0004	0,0005	0,0007		0,0013	0,0007	0,0006	0,0005
Q. look	0	0,0014	0,0015	0,0014	0,0014	0,0014	0,0019	0,0018	0,0021		0,0011	0,001	0,0009
Q. suber	0,0018	0,0011	0,0011	0,0013	0,0011	0,0011	0,0012	0,0011	0,0015	0,0025		0,0007	0,0006
Q. acutissima†	0,0016	0,0008	0,0008	0,001	0,0008	0,0008	0,0009	0,0009	0,0013	0,0022	0,0019		0,0005
Q. variabilis†	0,0008	0,0003	0,0003	0,0005	0,0003	0,0003	0,0004	0,0004	0,0007	0,0017	0,0014	0,0012	

2

3

4

Table 4(on next page)

Diversity values of the 5S IGS clones in the investigated dataset.

N: number of individuals; Cs: number of clone sequences; L: Aligned length (bp); O: occurrence of the IGS variants (u: unique, i: intra-individually identical; a: intraspecifically shared; s: interspecifically shared); D: distribution of the interspecifically shared variants (no. of variants); *p*: uncorrected *p*-distance range (STD); C: clusters identified with the Splitstree analysis; * including one putative hybrid with *Q. brantii*; ** including putative hybrids with *Q. suber* and *Q. libani.*

1

Dataset	N	Cs	L	O (u/i/a/s)	D	*p*	C
West Eurasian Cerris oaks	194	856	427	457/186/121/79		0,000 – 0,209 ± 0,021	1-4
Q. afares	5	17	379	10/2/5/0		0,000 – 0,019 ± 0,006	1
Q. brantii	7	26	403	9/11/4/2	*Q. cerris* (3)/*Q. look*, *Q. suber* (1)	0,000 – 0,088 ± 0,014	4
Q. castaneifolia	2	2	375	2/0/0/0		0,005 ± 0,003	2
Q. cerris	48	207	392	157/21/24/5	*Q. brantii* (1), *Q. trojana* (1), *Q. suber* (1)	0,000 – 0,202 ± 0,02	1[1], 2
Q. crenata	6	29	387	19/4/2/4	*Q. suber* (65)	0,000 – 0,054 ± 0,012	2[2], 3[3]
Q. libani	5	20	382	13/3/4/0		0,000 – 0,040 ± 0,009	1, 2[4]
Q. look	3	14	383	10/3/0/1	*Q. brantii*/*Q. cerris* (3)	0,000 – 0,032 ± 0,009	2
*Q. macrolepis**	28	158	402	44/71/43/0		0,000 – 0,065 ± 0,013	4
Q. ithaburensis	5	21	388	15/6/0/0		0,000 – 0,079 ± 0,014	4[5]
Q. suber	38	153	385	30/48/8/67	*Q. brantii* (1), *Q. cerris* (1), *Q. crenata* (4)	0,000 – 0,168 ± 0,019	1[6], 3
*Q. trojana***	43	192	391	130/30/31/1	*Q. cerris* (1)	0,000 – 0,198 ± 0,020	1, 2, 3[7]
Q. euboica	4	17	382	17/0/0/0		0,000 – 0,059 ± 0,012	2

2 [1] sample ce50 (S Italy), [2] sample cr04 (Slovenia), [3] including odd-placed sample cr05 (Croatia), [4] sample li01 (S Turkey), [5] including odd-

3 placed sample it03 (Israel), [6] sample su09 (S Spain), [7] sample tj08 (Botanical Garden of Naples)

4

Table 5(on next page)

Heatmap with the mean estimates of evolutionary divergence of the nuclear 5S IGS over sequence pairs within and between the investigated taxa.

Standard error estimates are shown above the diagonal.

1

Dataset	Intra-	Intergroup divergence											
Q. afares	0,0057		0,0065	0,0059	0,0058	0,0048	0,006	0,0064	0,0058	0,0062	0,0085	0,0052	0,0049
Q. brantii	0,0357	0,0353		0,0068	0,0066	0,006	0,0067	0,0044	0,0063	0,0068	0,0054	0,006	0,0061
Q. cerris	0,0167	0,0244	0,0427		0,0042	0,0043	0,0021	0,0062	0,0046	0,0021	0,0088	0,0056	0,0032
Q. castaneifolia	0,0053	0,0169	0,0343	0,0166		0,0048	0,004	0,0062	0,0045	0,0041	0,0085	0,0055	0,0035
Q. crenata	0,023	0,0241	0,0422	0,0266	0,0216		0,0044	0,0056	0,005	0,0046	0,0078	0,0028	0,004
Q. euboica	0,0194	0,026	0,0436	0,0193	0,0171	0,0285		0,0061	0,0046	0,0026	0,0088	0,0055	0,0031
Q. ithaburensis	0,0367	0,0364	0,0386	0,0423	0,0344	0,0422	0,0431		0,0057	0,0062	0,0056	0,0058	0,0056
Q. libani	0,0166	0,0232	0,0382	0,0244	0,0179	0,028	0,0255	0,037		0,0046	0,0078	0,0054	0,0041
Q. look	0,0134	0,023	0,0406	0,0159	0,0139	0,0255	0,0175	0,0401	0,0214		0,0089	0,0057	0,0032
Q. macrolepis	0,0194	0,0399	0,036	0,0498	0,0399	0,0473	0,0507	0,0376	0,0434	0,0481		0,0079	0,0079
Q. suber	0,0135	0,0211	0,0382	0,0279	0,0201	0,0197	0,0288	0,0385	0,0262	0,026	0,0423		0,0045
Q. trojana	0,027	0,0257	0,0433	0,0259	0,0195	0,0304	0,0266	0,043	0,0259	0,0235	0,0492	0,0287	

2

Figure 1

Median joining network of the *trnH-psbA* sequences in western Eurasian section *Cerris*.

Taxa are indicated with colours (see also File S1); black = Asian species of section *Ilex*; white = eastern Eurasian species of section *Cerris*. Line thickness according to 1, <5 and >5 mutations; * = shared with Asian *Ilex* oaks; ** = shared with Cerris-Ilex lineage of section *Ilex*; *** = shared with West-Med lineage of section *Ilex*; L1, L2 = haplotype lineages identified. All accession numbers are reported in supplementary Files S1 and S2.

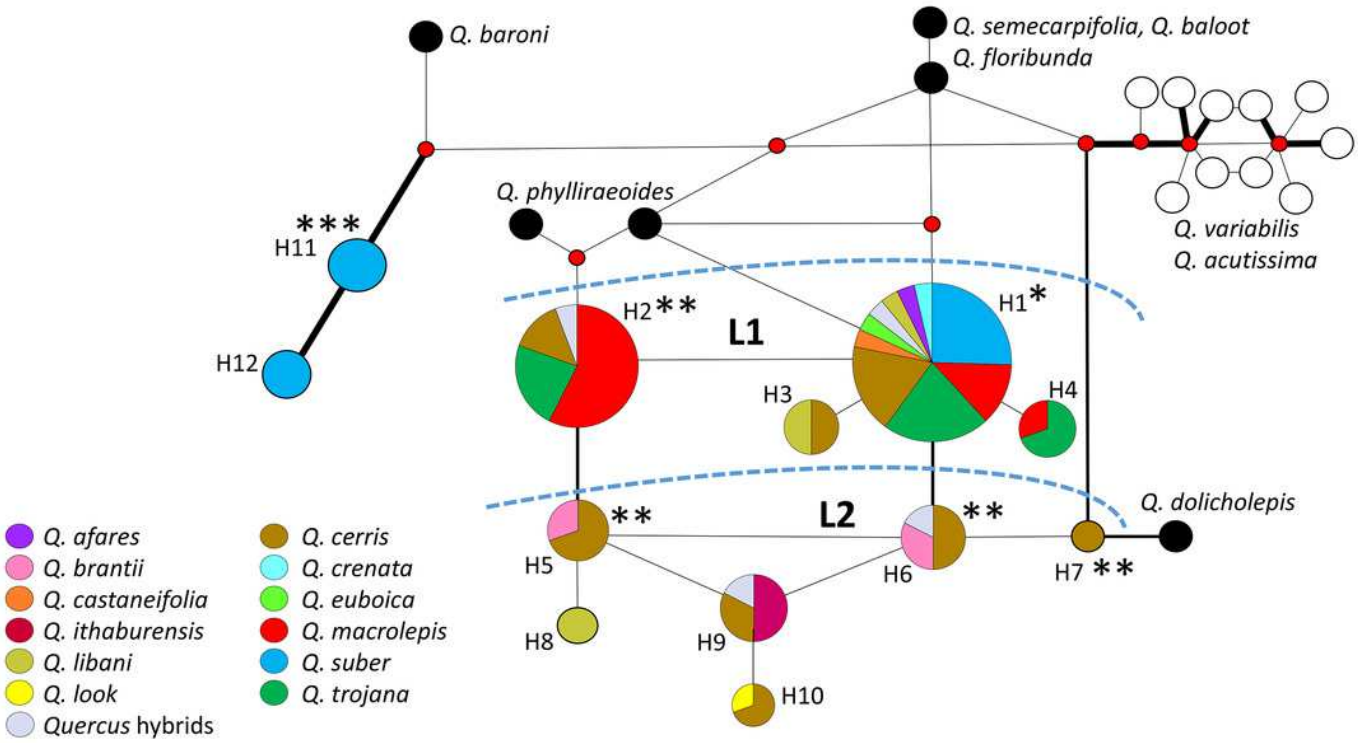
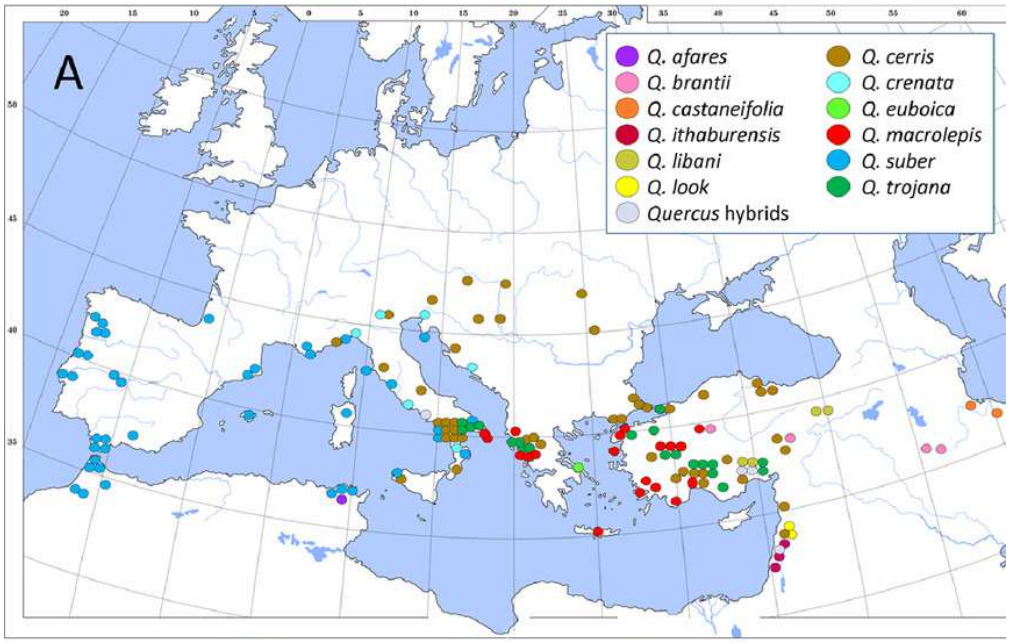


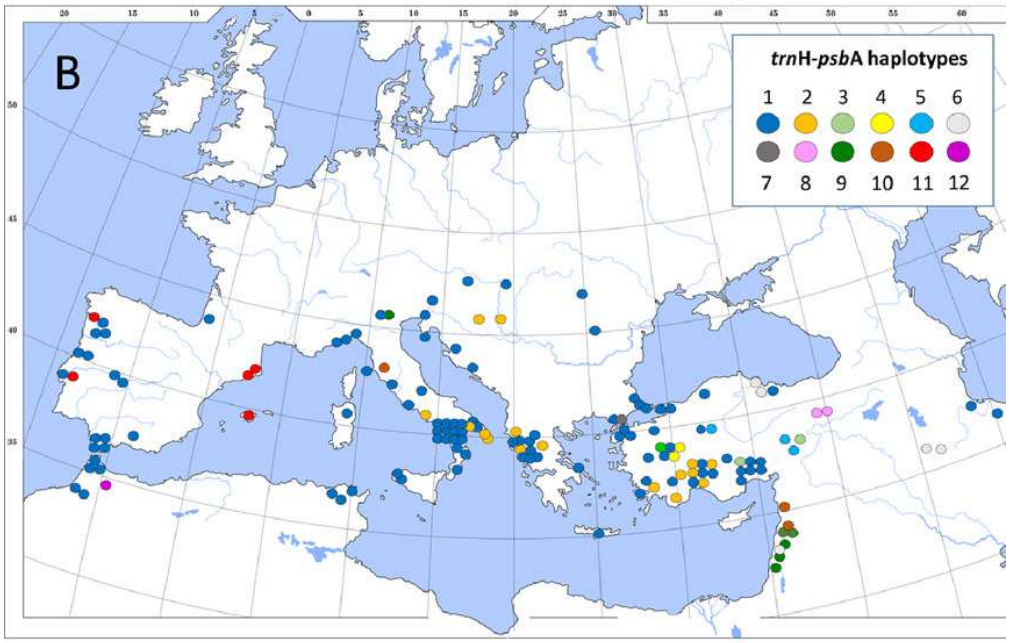
Figure 2

Geographic representation of the investigated dataset and its molecular signatures.

(A) sample distribution. (B) *trnH-psbA* haplotypes. (C) 5S-IGS clusters; see also supplementary File S1.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.26995v1 | CC BY 4.0 Open Access | rec: 20 Jun 2018, publ: 20 Jun 2018





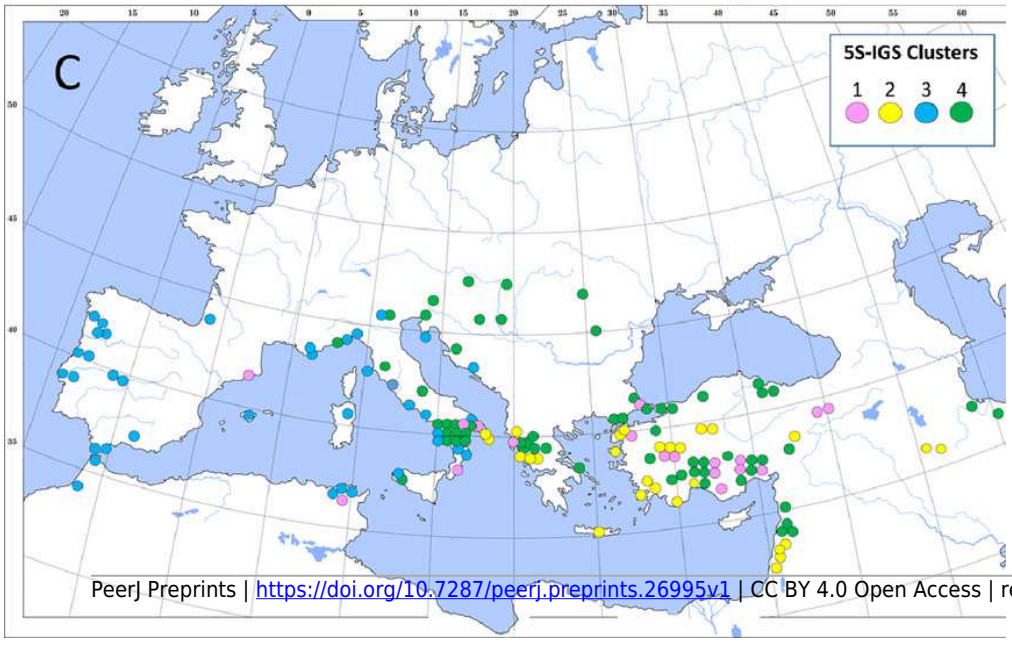


Figure 3

5S-IGS Clone-based RaxML tree.

The tree was rooted on *Q. baloot* and *Q. floribunda*. Taxa colors as in Fig. 1, 2a and File S1. Numbers 1-4 indicate the four major clades identified. Branch bootstrap support (1-100) is scaled as circles of increasing size (see also File S4 for details on clone labels and bootstrap values).

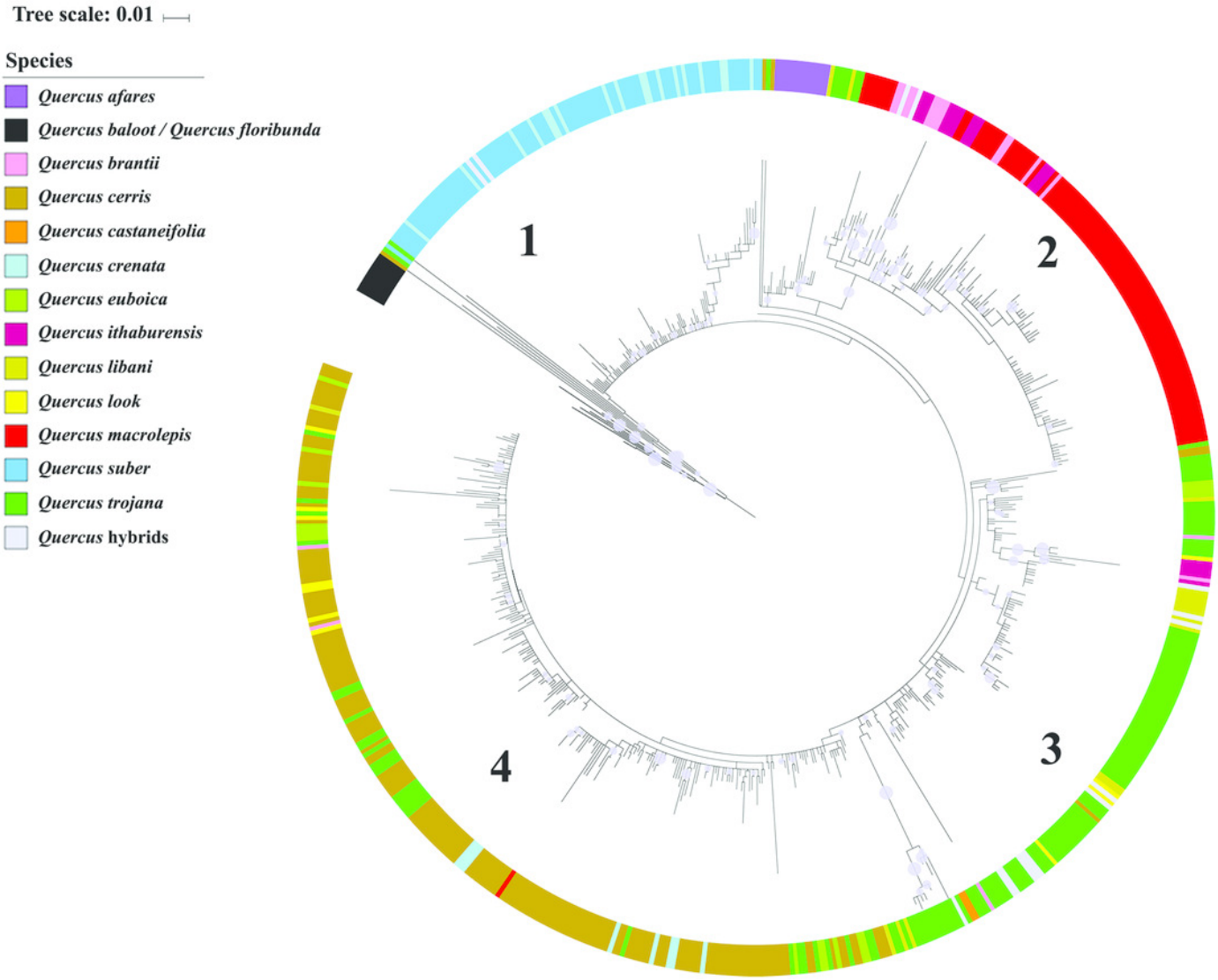


Figure 4

Network based on transformed 5S-IGS data showing inter-individual average (AVG) clonal distance relationships.

Only individuals represented by more than four clones are included (reconstructions for other cut-offs, m = 2, 3, or 5, are included in the Supplemental File S5; see also our Online Supplementary Archive).

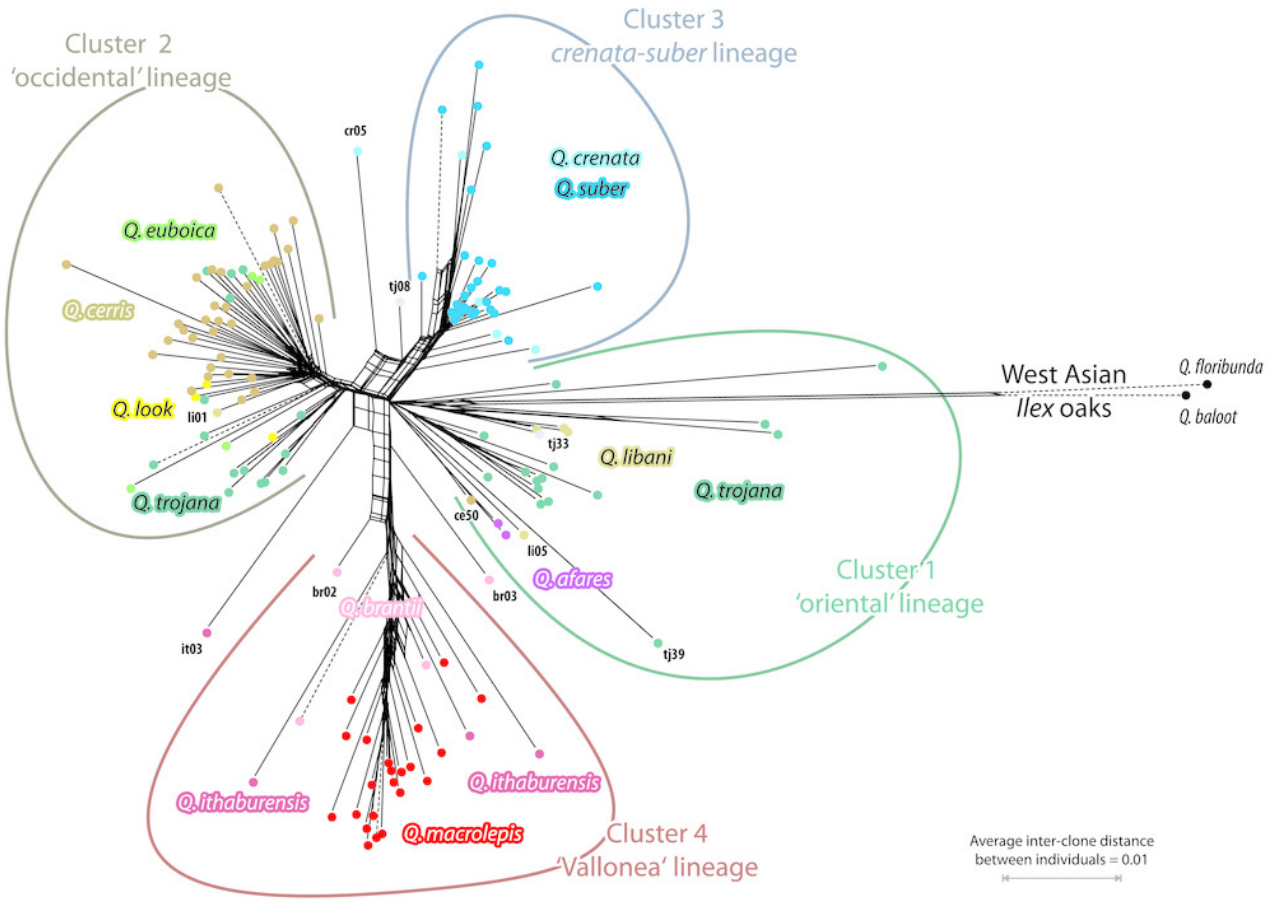


Figure 5

Network based on transformed 5S-IGS data showing inter-individual PBC clonal distance relationships.

Only individuals represented by more than four clones are included (reconstructions for other cut-offs, m = 2, 3, or 5, are included in our Online Supplementary Archive).

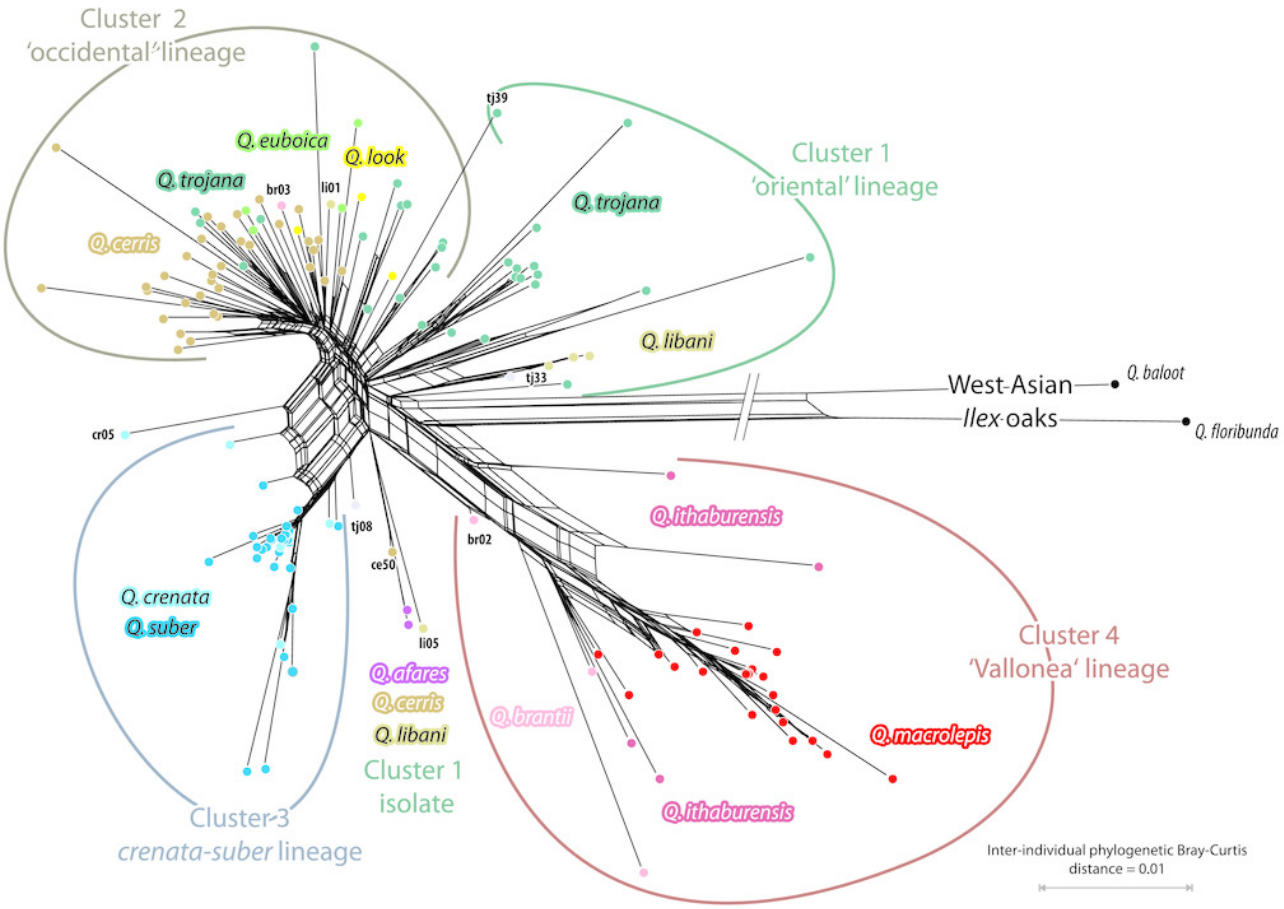


Figure 6

Mixed branching silhouette-tree doodle depicting the molecular differentiation and evolution in *Quercus* Section *Cerris*.

The evolutionary or genealogical lineages are indicated by branches (accordingly labelled and coloured), the fields represent shared or unique gene pools. The deep incongruence between plastid genealogies and nuclear-morphological phylogenetic lineages can only be explained by ancient reticulation and incomplete lineage sorting during the formation and isolation of the modern-day lineages following the break-up of the ancestral gene pool (tentatively labelled as 'proto-*Ilex*').