# Supplementary Data 4

This file includes supplementary background information and additional results on molecular and morphological diversity found in species of *Quercus* section *Cerris.*

Introduction into Quercus *section* Cerris

Quercus section *Cerris* Dumort. (Denk *et al.*, 2017*a*) is a small group of oaks of the Eurasian subgenus *Cerris* Oerst., which includes the iconic cork oak, *Q. suber* L., the source of commercial cork. Well-developed cork layers are present not only in the western Mediterranean *Q. suber* but also in the East Asian *Q. variabilis* Blume ("Chinese cork oak"). Both are used industrially (Huang *et al.*, 1999). Clearly developed but much thinner cork layers occur in *Q. crenata* Lam., *Q. afares* Pomel, and some varieties and forms of *Q. cerris* L. (for example var. *gussonei* Borzì in Sicily; "subsp./var. *pseudocerris*" in Turkey and Lebanon; Maire, 1961; Schirone *et al.*, 2015; Pignatti, 2017–2019). Distinctly corky bark does not occur in any other oak section, but it is present in at least five out of the 15 species included in section *Cerris* and justifies the name "cork oaks" for the section.

The cork oak section is remarkable biogeographically and ecologically. First, it is the only group of oaks that is most diverse in western Eurasia, despite also being widespread in East Asia (Supplementary Data 3). Second, it is strictly deciduous but comprises several semi-evergreen species adapted to summer-dry and winter-cold, continental climates. Third, a single species in this group, *Q. cerris*, the most widespread western Eurasian cork oak, independently evolved lobed leaves reminiscent of many species in *Quercus* section *Quercus*, the white oaks. Finally, the cork oaks provide well-documented examples of intrasectional and intersectional introgressive hybridization that provide insight into the timing of diversification and gene flow in the genus. In a recent study, Simeone *et al.* (2018) investigated western Eurasian members of section *Cerris* using a broad-sampled chloroplast (*trnH-psbA*) and a multi-copy nuclear (5S-IGS) marker. Chloroplast haplotypes reflected shared biogeographic histories of all western Eurasian *Cerris* species and its sister section *Ilex*, indicating several phases of chloroplast capture and secondary, species-decoupled, geographic sorting. Simeone *et al.* (2018) suggested that the precursors of western Eurasian *Cerris* species came into contact with *Ilex* only after the initial range expansion of *Cerris* from north-eastern Asia during the early Oligocene. In addition, four main nuclear 5S-IGS lineages were identified within *Cerris*, three of which were private to potential sibling species: (*i*) root-proximal 'Oriental' lineage in *Q. afares*, *Q. libani*

Olivier, *Q. trojana* Webb; (*ii*) 'Occidental' lineage found in the widespread *Q. cerris* and the narrow-endemics *Q. castaneifolia* C.A.Mey. (northern Iran), *Q. look* Kotschy (Levant), and *Q. euboica* (Papioann.) K.I.Chr. (Euboea), and as intra-individual dimorphism in western *Q. trojana*; (*iii*) 'Vallonea' lineage exclusive to the eastern Mediterranean *Q. brantii* Lindl., *Q. ithaburensis* Decne., and *Q. macrolepis* Kotschy; and (*iv*) a derived lineage characterising the western-most species pair *Q. crenata-Q. suber* (Fig. S4-1).
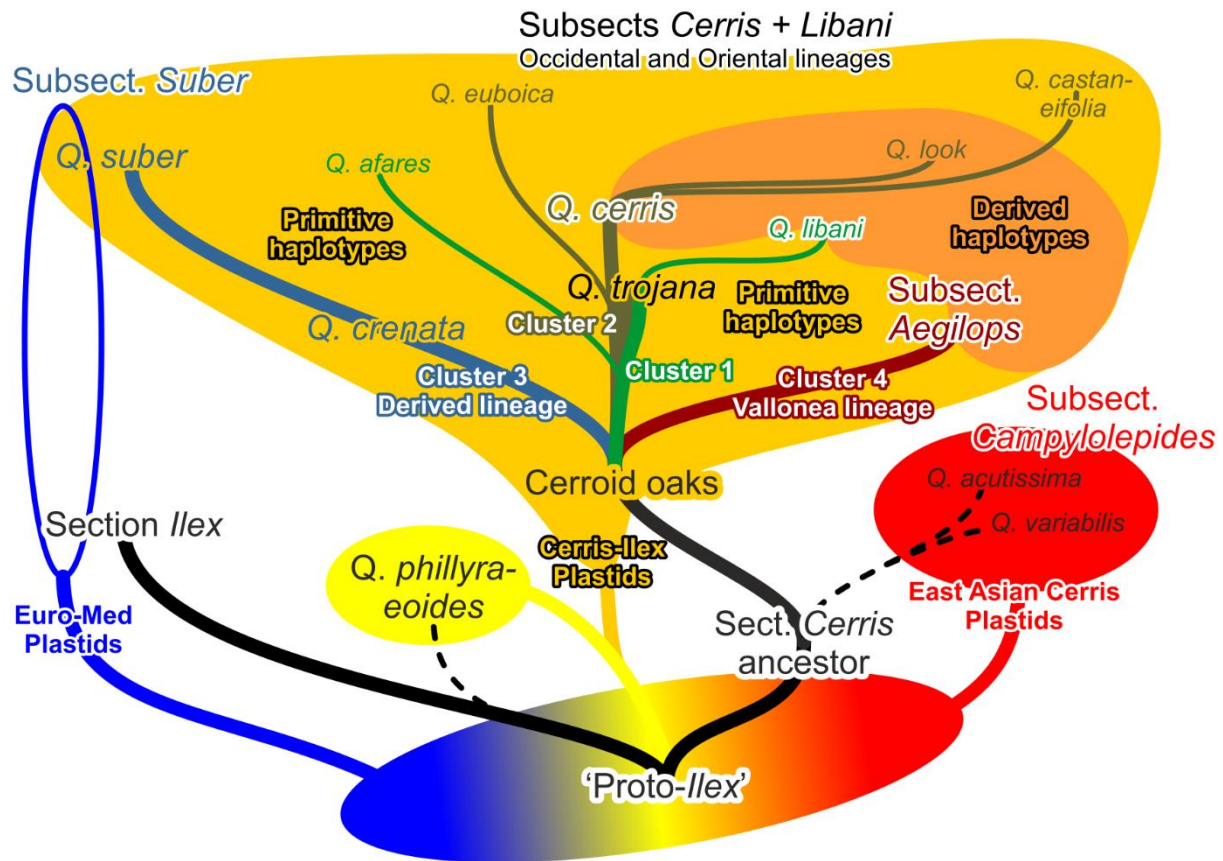

Figure S4-1. Cactus-type tree metaphor summarizing putative reticulate-evolutionary pathways in western Eurasian species of section *Cerris* (modified after Simeone *et al.*, 2018, fig. 6). Lines indicate major nuclear (5S-IGS) lineages, coloured fields shared plastid (*trnH-psbA*) haplotypes.

Taxonomy and previous schemes

Some of the clades recovered from the RAD-seq data in the present study have previously been recognized as infra-sectional groups. Camus (1936–1938) united the East Asian members of section *Cerris* within her subsection *Campylolepides*. *Quercus suber* has previously been accommodated within its own subsection *Suber* (Menickij, 1984). Nevertheless, the majority of clades recognized here do not correspond to previously recognized infrageneric taxa (main-text Table 2) but agree with the 5S-IGS-defined species groups in Simeone *et al.* (2018) and are here treated as subsections (Fig. S4-1). The discrepancy between morphological groupings recognized previously and the clades recognized in this study

on molecular grounds may represent a combination of convergence (parallelism; Fig. S4-2, Table S4-1) among clades and ecological divergence within clades.
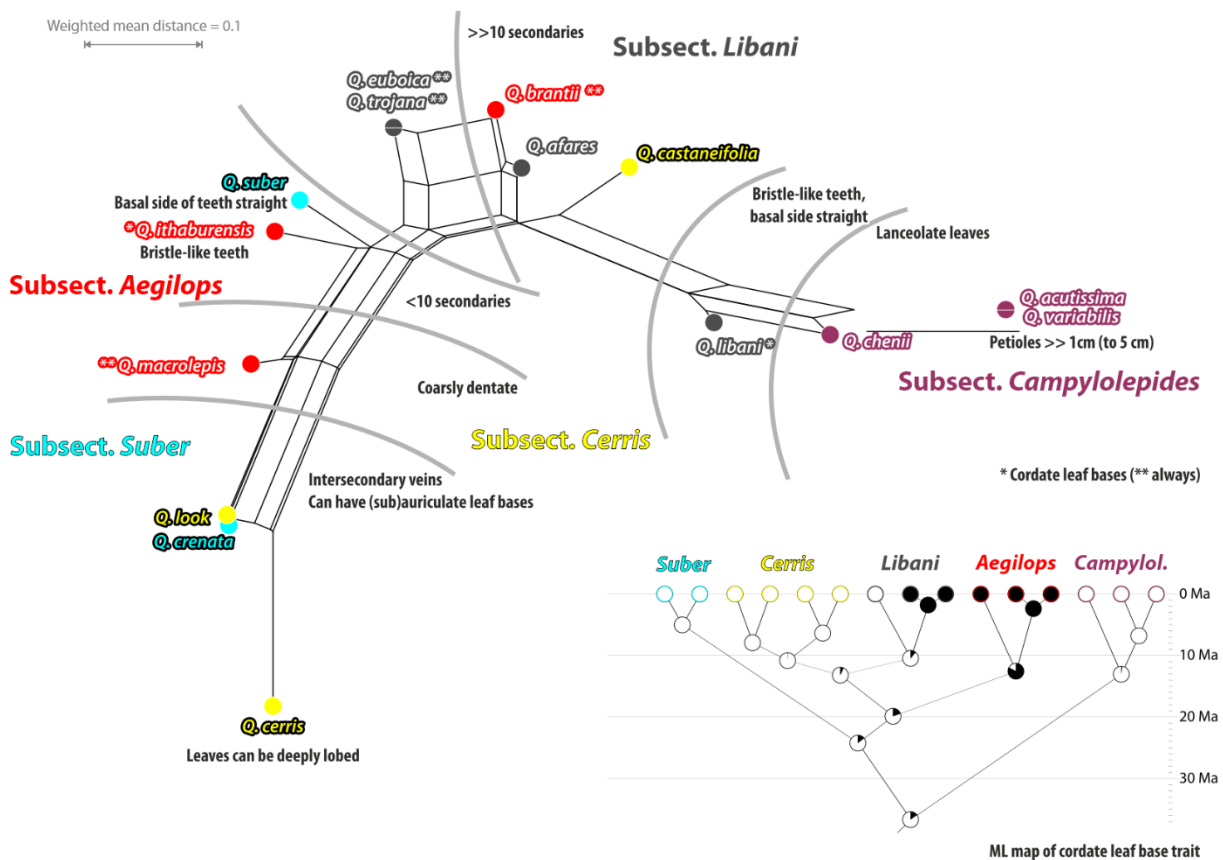
Figure S4-2. Neighbour-net splits graph inferred from pairwise (weighted) distances calculated from a leaf-morphological matrix (included in Supplementary Data 4; cf. Table S4-1). Note the poor phylogenetic sorting (subsections = molecular clades inferred based on nuclear data) of leaf-morphological groups (species being part of the same neighbourhood) in spite of the relative tree-like graph. Bottom left: Maximum likelihood mapping of the cordate leaf base trait on the dated phylogeny. For terminology, see Figure S4-3.

Subsect. *Suber*—Although the cork oak *Q. suber* is generally considered an evergreen tree, and therefore has been associated with section *Ilex* by Menickij (1984), it behaves differently from fully evergreen oak species. Its leaves are thinner and there is a tendency to shed most of the leaves in spring or early summer as response to early moisture stress, giving the tree a pronounced deciduous aspect (Schwarz, 1936–39; Escudero and del Arco, 1987; le Hardÿ de Beaulieu and Lamant, 2010). Menickij (1984) speculated (translation of the Russian original 2005, p. 444) "*The only species of the subsection* Suber – *the Mediterranean* Q. suber – *gives the impression of an intersectional hybrid between some initial species of the preceding subsection* [the subsection *Heterobalanus*; = sect. *Ilex*, the Ilex oaks] *and species of the* Q. aegilops *group (section* Cerris*).*" Indeed, *Q. suber* has a number of highly specialized ecological adaptations. Most conspicuous is the thick cork layer of the stem, which protects the

3

trees from wildfires. Further, the sudden abscission of leaves in dry periods is an effective adaptation to seasonal dry climates, and the plastic cycle of fruit maturation may be a response to changeable availability of nutrients and water. A thinner cork layer is also produced by *Q. crenata*, which our analysis resolves as the only other member of the clade comprising *Q. suber*. This cork layer has been one reason for assuming that *Q. crenata* was a hybrid between *Q. suber* and *Q. cerris* (Pignatti, 1982; Schwarz, 1936–1939). In sum, the now available data point to an ancestor-descendant relationship, with *Q. suber* representing a highly adapted species that may have evolved from a *Q. crenata*-like ancestor (cf. main-text Fig. 1; Supplementary Data 3; Simeone *et al.*, 2018, figs 5–6). Plate S3-1 illustrates the ancestral-primitive (plesiomorphic) character of *Q. crenata* leaves in comparison to one of the earliest and widespread leaf fossils that can be assigned to section *Cerris*: *Q. kraskinensis* Pavlyutkin. The disparate partitioned *D*-statistics results (Supplementary Data 1) could result from an ancestor-descendant relationship. Being a recent and strongly bottlenecked offshoot of the subsection *Suber* lineage, we find no evidence for past or recent admixture in the *Q. suber* individuals from outside the lineage. The statistically significant *D*-values observed for the *Q. crenata* individuals may reflect retained ancestral polymorphism inherited from the subsection's precursors as well as a higher susceptibility for introgression of this less evolved and niched sibling (Table S1-1 in Supplementary Data 1; main-text Fig. 4).

Subsect. *Aegilops*—The 'Vallonea-oaks', subsection *Aegilops*, share several morphological similarities (Fig. S3-3; Avishai, 2016). The definition of the Vallonea-oaks has changed through time (main-text Table 2). The concept of subsect. *Aegilops* proposed here, including only *Q. brantii*, *Q. ithaburensis*, and *Q. macrolepis* (see also Schwarz, 1936), is supported both by our RAD-seq dataset and by 5S-IGS differentiation patterns (Denk and Grimm, 2010; Simeone *et al.*, 2018). The species share unique 5S-IGS variants not found in any other species of section *Cerris*. Their nuclear SNP patterns show a high coherence (well-developed, unambiguously supported root branch despite substantial tip divergence, main-text Fig. 1).
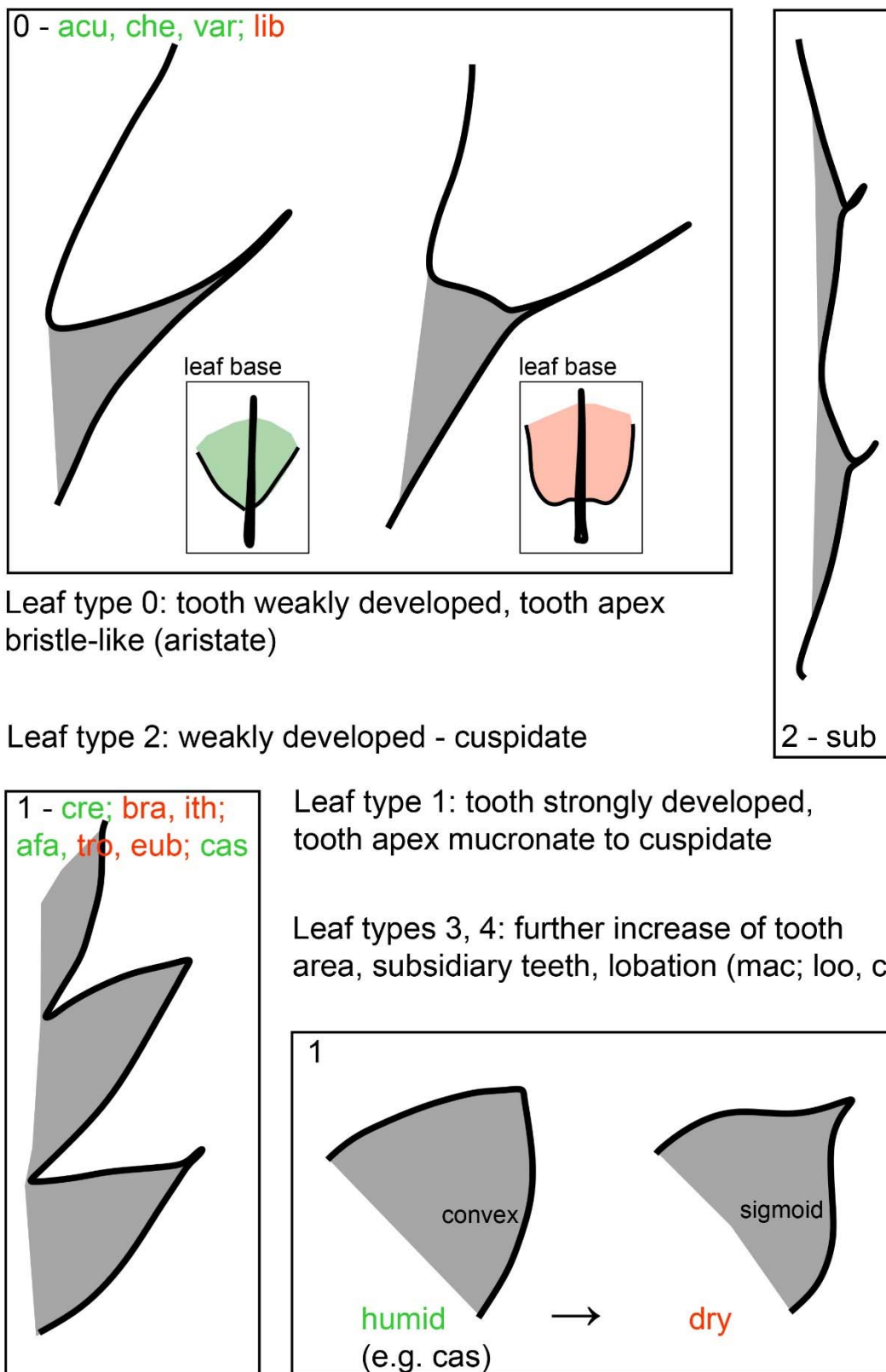
Subsection *Libani*—Ørsted (1871) and later Schwarz (1936) and Menickij (1984) united the East Asian and western Eurasian fully deciduous species of section *Cerris* with a prominent mucronate leaf margin (Fig. S3-2) in the same section or subsection. Likewise, most monographers considered *Q. afares* and *Q. castaneifolia* as belonging to the same section/subsection (main-text Table 2), associations that can be rejected based on nuclear data (Simeone *et al.*, 2018; this study). In our analysis, *Q. afares* is resolved in a clade together with

Q. libani and *Q. trojana*; these three species share a lineage of 5S-IGS variants ('Oriental lineage', Cluster 1 in Simeone *et al.*, 2018; cf. Fig. S3-1) not found in subsection *Cerris* as defined below. As in the case of nuclear-encoded ribosomal spacer data (Denk and Grimm, 2010; Simeone *et al.*, 2018), we found no evidence for a white oak parentage in *Q. afares* (see also Supplementary Data 1).

Subsect. *Cerris*—Schwarz (1936) suggested a close relationship between the geographically isolated and ecologically narrow *Q. castaneifolia* and *Q. cerris.* Our data demonstrate that the narrow endemic *Q. look*, traditionally associated with the Vallonea-oaks (subsect. *Aegilops*), also belongs to subsection *Cerris*. As seen in the full-tip ML tree (main-text Fig. 1), the genetic variation found in *Q. cerris* encompasses its two endemic siblings, in striking analogy to earlier 5S-IGS data ('Occidental lineage', Cluster 2 in Simeone *et al.*, 2018). Thus, subsect. *Cerris* represents a case example for the retention of ancestral morphologies in locally isolated populations leading either to the formation of relict species (*Q. castaneifolia*) or to highly adapted (*Q. look*) Mediterranean species. Further in-depth nucleome analyses are likely to reveal cryptic or at least previously unrecognized species in *Q. cerris* and/or recurrent ancient hybridisation events in subsection *Cerris*, probably involving members of subsection *Libani* (5S-IGS dimorphism in *Q. trojana*; Simeone *et al.*, 2018; but very low *D*-values, ≤89% of the tests statistically significant for introgression from *Q. cerris* into *Q. trojana*).

Species not yet studied—The only section *Cerris* species not included in the present study, *Q. euboica*, has recently been investigated using one chloroplast and one nuclear marker (Simeone *et al.*, 2018). Traditionally considered a subspecies of *Q. trojana*, the only species of section *Cerris* with individuals showing a prominent 5S-IGS dimorphism, *Q. euboica* appeared intermediate between *Q. cerris* and *Q. trojana* (Fig. S3-2). Thus, *Q. euboica* clearly is a member of the *Cerris* core group established here. Based on the available molecular data, its taxonomic placement remains ambiguous, belonging either to subsection *Libani* or *Cerris*, in analogy to the geographic isolates *Q. castaneifolia* and *Q. look*. Further in-depth studies are needed to assess the amount of secondary homogenisation because of sub-recent hybridisation with or without introgression by *Q. cerris*.

0 - acu, che, var; lib

leaf base

leaf base

Leaf type 0: tooth weakly developed, tooth apex bristle-like (aristate)

Leaf type 2: weakly developed - cuspidate

2 - sub

1 - cre; bra, ith; afa, tro, eub; cas

Leaf type 1: tooth strongly developed, tooth apex mucronate to cuspidate

Leaf types 3, 4: further increase of tooth area, subsidiary teeth, lobation (mac; loo, cer)

1

convex

sigmoid

humid (e.g. cas) → dry

Figure S4-3. Classification of general leaf types found in section *Cerris* introducing the main discriminative features scored for the morphological matrix included in Supplementary Data 4 (cf. Table S4-1). Specific character suites (see also Fig. S4-2) were used to assign leaf fossils to clades to date the phylogeny (see main text). Species names are abbreviated by the first three letters.

Table S4-1. Leaf traits scored for the morphological matrix included in Supplementary Data 4.

Character	[1]	1	2	[3]	3	4	[5]	5
Species	[Leaf size (length, cm)]	Leaf size	Leaf organisation	Secondary veins	Secondary veins	Intersec. Veins	[Petiole]	Petiole
Q. acutissima	8-19	**Large**	Simple	13-18	>>10	Absent	>>1cm (to 5 cm)	**Long**
Q. chenii	7-12	Medium	Simple	12-16	>>10	Absent	1cm (to 1.5 cm)	Short
Q. variabilis	8-15(-20)	**Large**	Simple	13-18	>>10	Absent	>>1cm (to 5 cm)	**Long**
Q. crenata	4-9	Medium	Simple	5-8	<10	**Present**	1-1.5 cm	Short
Q. suber	3-7	Small	Simple	5-6	<10	Absent	0.5-1 cm	Short
Q. brantii	6-10(-13)	Medium	Simple	8-14	>>10	Absent	0.5-2 cm	Short
Q. ithaburensis	4-9	Medium	Simple	5-8	<10	Absent	1-2 cm	Short
Q. macrolepis	5-9	Medium	Simple	5-8	<10	Absent	1-3.5 cm	Medium
Q. afares	7-14	Medium	Simple	8-17	>>10	Absent	1-2 cm	Short
Q. libani	7-12	Medium	Simple	11-16	>>10	Absent	0.8-2 cm	Short
Q. trojana	3-8(-10)	Medium	Simple	9-10	c.10	Absent	0.5-1 cm	Short
Q. euboica	5-9(-11)	Medium	Simple	8-10	c.10	Absent	0.5-1 cm	Short
Q. castaneifolia	10-20	**Large**	Simple	8-14	>>10	Absent	1-2.5 cm	Short
Q. look	5-7.5	Small	Simple	4-8	<10	**Present**	0.5-1.5 cm	Short
Q. cerris	5.5-14(-20)	**Large**	**Simple to deeply lobed**	5-9	<10	**Present**	0.3-2cm	Short

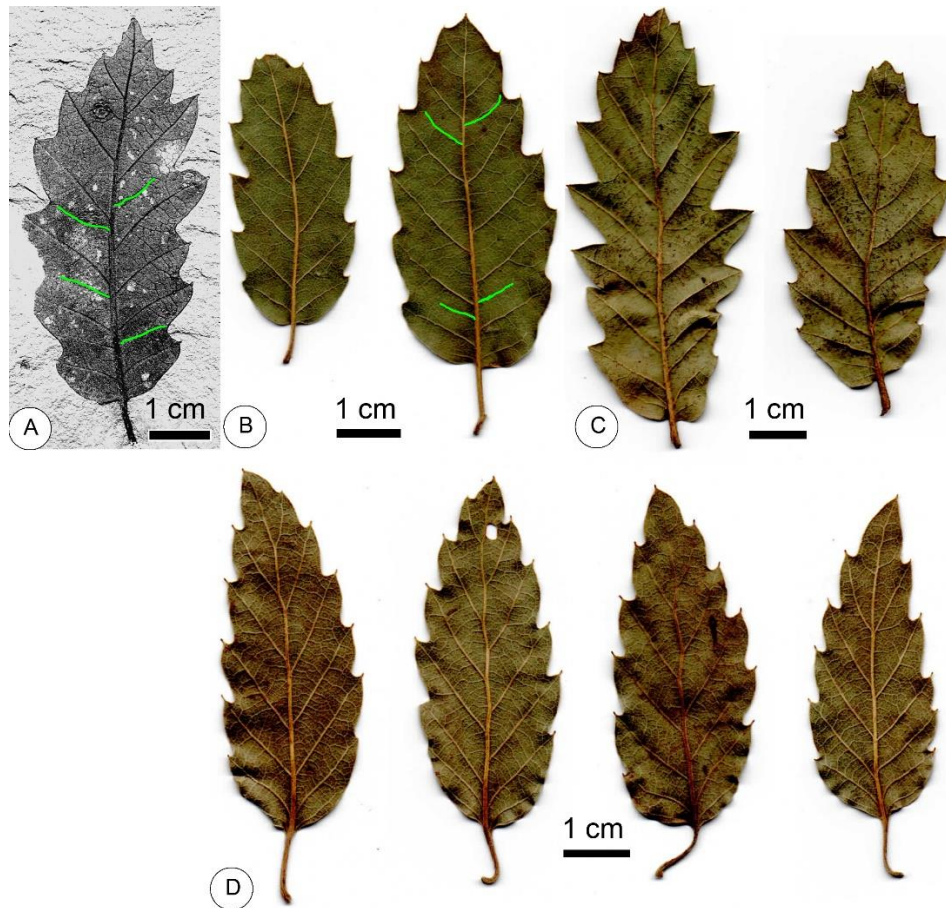| Character	6	7	8	9	10	
Species	Leaf base	Leaf shape	Leaf margin	Tooth type	Basal tooth side	Tooth type categories
Q. acutissima	Obtuse, acute	**Narrow ovate, lanceolate**	Dentate	**Bristle-like**	**Weakly developed, Straight**	plesiomorph 1	0
Q. chenii	Obtuse, acute	**Narrow ovate, lanceolate**	Dentate	**Bristle-like**	**Weakly developed, Straight**	plesiomorph 1	0
Q. variabilis	Obtuse, acute	**Narrow ovate, lanceolate**	Dentate	**Bristle-like**	**Weakly developed, Straight**	plesiomorph 1	0
Q. crenata	Obtuse, acute, subauriculate	Narrow ovate, elliptic	Coarsely dentate	Coarse triangular	Convex to sigmoid	plesiomorph 2	1
Q. suber	Obtuse, acute	Ovate, elliptic	Dentate	Cuspidate	**Weakly developed**	derived 1 sclerophyllous	2
Q. brantii	Cordate	Ovate, elliptic	Dentate	Cuspidate	Convex to sigmoid	plesiomorph 2	1
Q. ithaburensis	Obtuse to cordate	Ovate, elliptic	Dentate	Cuspidate to **bristle-like**	Convex to sigmoid	plesiomorph 2	1
Q. macrolepis [incl. Q. vallonea]	Cordate	Ovate to broad ovate	Coarsely dentate	Coarse triangular	Convex	derived 2	3
Q. afares	Obtuse, acute	Narrow ovate, elliptic	Dentate	Coarse triangular to cuspidate	Convex to sigmoid	plesiomorph 2	1
Q. libani	Cordate, obtuse	Narrow ovate, elliptic, oblong	Dentate	**Bristle-like**	**Weakly developed, Straight**	plesiomorph 1	0
Q. trojana	Cordate	Narrow ovate, elliptic, oblong	Dentate	Cuspidate	Convex to sigmoid	plesiomorph 2	1
Q. euboica	Cordate	Narrow ovate, elliptic	Dentate	Cuspidate	Convex to sigmoid	plesiomorph 2	1
Q. castaneifolia	Obtuse, acute	Narrow ovate, elliptic	Coarsely dentate to dentate	Coarse triangular to cuspidate	Convex to sigmoid	plesiomorph 2	1
Q. look	Obtuse, acute, (sub)auriculate	Narrow ovate, elliptic	Coarsely dentate	Coarse triangular	Convex to sigmoid	derived 2	3
Q. cerris	Obtuse, acute, (sub)auriculate	Narrow to broad elliptic, obovate	Coarsely dentate	Coarse triangular	Convex	derived 3	4

Ad char. 10: Weakly developed = tooth area close to 0, tooth reduced to cusp or bristle-like extension (see **Fig. S4-3**).

Abbreviations used in legend (Fig. S4-3): acu – *Quercus acutissima* , afa – *Q. afares* , bra – *Q. brantii* , cas – *Q. castaneifolia* , cer – *Q. cerris* , che – Q. chenii, cre – *Q. crenata* , eub – *Q. euboica* , ith – *Q. ithaburensis* , lib – Q. libani, loo – *Q. look* , mac – *Q. macrolepis* , sub – Q. suber, tro – *Q. trojana* , var – *Q. variabilis* .

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A. Q kraskinensis Pavlyutkin **FOSSIL**

B. Q. crenata Lucombeana002

C. Q. crenata Valentano0003

D. Q. crenata Tassarolo001

E. Q. crenata
Santi 1795 [Q pseudo robur]

/ = intersecondary veins

Plate S4-1. Leaf morphologies shared by modern-day *Q. crenata* and fossil-species *Q. kraskinensis* (Oligocene to Miocene, Central Asia to western Eurasia); representing a likely symplesiomorphic leaf type of section *Cerris.*