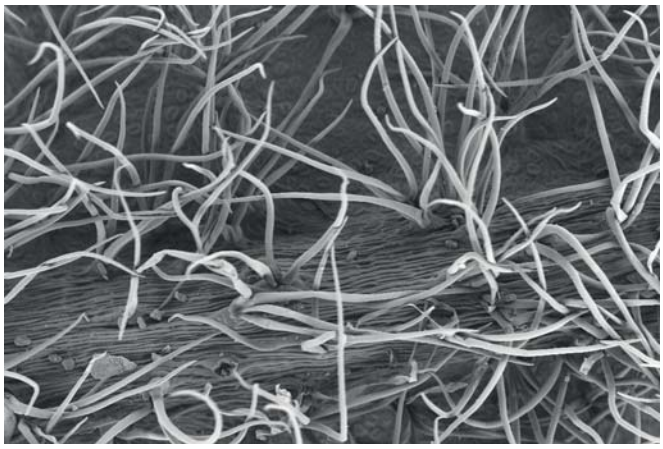

Figure 3/ *Quercus pyrenaica* has fasciculate, radiate, stipitate hairs (bar = 100 ηm; discussion, p. 125).

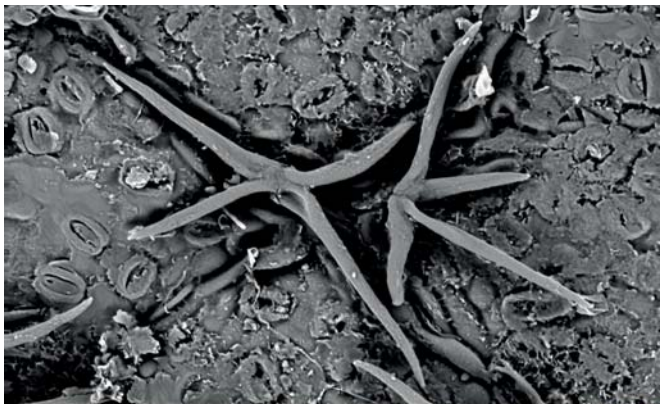

Figure 4/ *Quercus estremadurensis* shows uniseriate-branched, smooth hairs (bar = 30 ηm; discussion, p. 126).

Species/Hairs	Simple 1	Simple 2	Simple 3	Bulb	Branched	Fascic. 1	Fascic. 2	Radiate	Stellate	Rosulate
Q. faginea subsp. *alpestris*	-/-	-/+	+/+	+/-	-/-	-/+	+/+	-/-	+/+	-/+
Q. broteroi	-/-	-/+	+/+	+/-	-/-	-/+	+/+	-/+	+/+	-/+
Q. canariensis	-/+	-/+	+/+	-/+	-/-	+/+	+/+	-/-	-/+	-/-
Q. coccifera	-/-	-/+	-/+	+/-	-/-	-/-	-/-	-/-	+/+	-/-
Q. estremadurensis	-/+	-/+	+/+	+/+	-/+	-/+	+/+	-/-	-/+	-/-
Q. faginea	-/-	-/+	+/+	+/-	-/-	-/+	+/+	-/-	+/+	-/-
Q. lusitanica	-/-	-/+	+/+	+/-	-/-	-/+	+/+	-/+	+/+	+/+
Q. pyrenaica	-/-	+/+	+/+	+/-	-/-	+/+	+/-	-/-	+/+	-/-
Q. rotundifolia	-/-	-/+	+/+	+/-	-/-	+/+	+/+	-/+	+/+	+/+
Q. suber	-/-	-/+	+/+	+/-	-/-	-/+	+/+	-/-	+/+	-/-

Table 1/ Hair types and distribution on adaxial/abaxial leaf blade surfaces of Mediterranean oaks from SW Iberian Peninsula. Simple 1: Simple, uniseriate, smooth base; Simple 2: Simple, uniseriate, scaly base; Simple 3: Long, simple not uniseriate; Fascic. 1: Fasciculate, stipitate; Fascic. 2: Fasciculate, not stipitate (discussion, p. 125).

Micromorphologcal and Anatomical Characters Used to Differentiate Mediterranean Oaks

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ABSTRACT

This study discusses the foliage, floral and reproductive microcharacters used for the separation of species of the genus *Quercus* in the Mediterranean. Results for species of the SW Iberian Peninsula show the presence of some microcharacters such as foliar trichomes that allow the separation of taxa at a specific level. Other microcharacters such as the micromorphology of the stomata, the cells of the epidermis, the anthers, or pollen grains, make it possible to separate clearly the three subgenera represented in the territory: *Cerris* (Spach) Örsted, *Sclerophyllodrys* O. Schwarz and *Quercus*. Finally some typical microcharacters of *Quercus* species were found which had not been previously observed in oaks of the SW Iberian Peninsula, such as: verrucate stomata (*Q. faginea* subsp. *alpestris* (Boiss.) Maire), uniseriate-branched hairs (*Q. estremadurensis* O. Schwarz), and apiculate and pilose anthers (*Q. suber* L.)

Keywords: *Quercus*, stomata, trichome, anatomy, anther, pollen, micromorphology

Introduction

Taxonomic studies of the genus *Quercus* in the Western Mediterranean have relied mainly on visible morphological characters. Most authors of the 19th and 20th centuries used leaf blade and fruit characters, and to a lesser extent, those of flowers, to segregate and characterize different species of *Quercus* (Camus 1936-1954; Coutinho, 1888; De Candolle, 1868; Huguet de Villar, 1957; Schwarz, 1936, 1964).

In the mid-20th century, scientific advances in microscopic and ultra-microscopic equipment allowed for more detailed studies of pollen and leaf trichome morphology on which to base the segregation of *Quercus* species worldwide, especially in North America and Europe (Dyal, 1936; Hardin, 1976, 1979; Solomon, 1983a; 1983b; Stairs, 1964; Van de Campo and Elhai, 1956). The end of the past century marked the beginning of descriptive studies of the diversity of foliar trichomes of Western Mediterranean species of the genus *Quercus* (Bussotti and Grossoni, 1997; Llamas et al., 1995; Penas et al., 1994), and of pollen morphology (Colomo et al., 1983; Gomez-Casero et al., 2004; Ruiz del Castillo, 1988). The increase of descriptive studies of micromorphological characters of the pollen grain and of the leaf blade surface has provided a more accurate method of species segregation within the genus. However, some species of the Southwest Iberian Peninsula have not been studied and therefore the objective of this work is to increase knowledge of the micromorphology and anatomy of these species.

Methodology

Samples

Leaf samples were obtained from the HSS Herbarium (Holmgren et al., 2003), while the reproductive organ samples were collected April 10-26, 2012 in the *Quercus* collection of the Research Center La Orden.

Anatomical study

For leaf surface study, the samples underwent forced hydration for 4 hours in distilled water at 55 °C, and were subsequently prepared following the latest methodology described by Devesa (1992) for the study of the foliar epidermis in *Poaceae*.

Microscopic observation and photography were carried out with a LEICA DMBR model. The terminology used for the description of the samples is according to Jones (1986); Lou and Zhou, (2001); Liu et al., (2009).

SEM study

The pollen grains were investigated and documented by scanning electron microscopy (SEM, single grain technique by Hesse et al., 2009). SEM stubs with pollen are stored in the palynological collection of the Department of Forest Production, Research Center La Orden, (HSS-PAL) under the numbers 2554-2589. Terminology for pollen descriptions follows Punt et al., (2007); Hesse et al., (2009); Rowley & Gabarayeva (2004) and Denk and Grimm (2009); the trichome terminology used for classification is that suggested by Hardin (1976, 1979); Jones (1986); Llamas et al., (1995); Uzunova et al., (1997); and the terminology of Safou et al., (1988) is used for the stomata.

A ZEISS, EVO-10 model at 10-20 KeV was used for the SEM observations and photographs.

Presentation of results

The results are presented in a table and figures that allow the identification of the

characters mentioned in the text. Taxonomic treatment follows Amaral Franco (1990) and Nixon (1997).

Results and Discussion

Study of the leaf blade characters

1. Leaf anatomy

The study of the leaf epidermis shows two species groups: a) evergreen species with short, rounded cells and thick-walled cells, that includes species of the subgenera *Sclerophyllodrys* O. Schwarz and *Cerris* (Spach) Örsted (Figure 1); and b) deciduous species, with large, elongated cells and thin-walled cells, that includes species of subgenus *Quercus* (Figure 2).

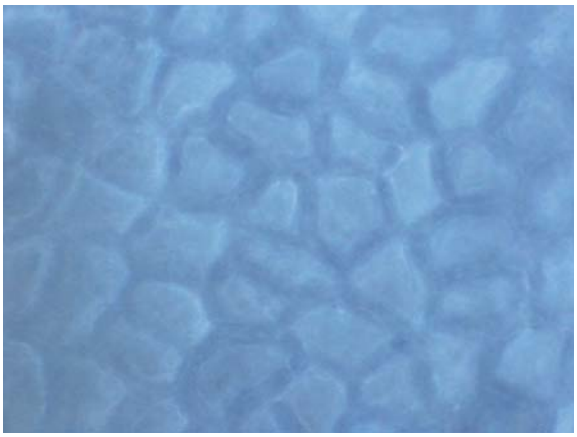

Figure 1/ Adaxial leaf blade epidermis of *Quercus suber* with rounded cells and thick-walled cells (bar = 450 ηm).

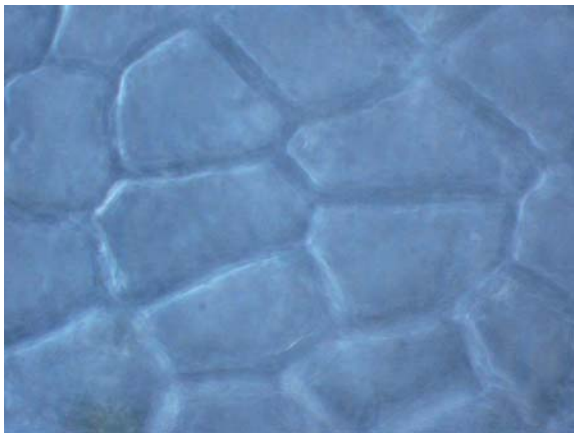

Figure 2/ Adaxial leaf blade epidermis of *Quercus broteroi* with elongated cells and thick-walled cells (bar = 450 ηm).

The cellular structure in the leaf blade epidermis is related to habitat. Taxa of arid zones, like the evergreen species from the SW Iberian Peninsula, have leaves with a thick cuticle and short, thick-walled cells. The taxa of humid habitats have thin cuticles and cells with long, thin walls, such as the deciduous species of our study (Clements, 1905; Hanson, 1917).

2. Trichomes

The study of foliar trichomes and their distribution in the leaf blade reveals the presence of species-characteristic trichomes. Rosulate-type trichomes appear in *Q. faginea* subsp. *alpestris* (Boiss.) Maire, *Q. broteroi* (Coutinho) Rivas-Martínez, *Q. lusitanica* Lam., and *Q. rotundifolia* Lam. All of these species belong to two of the subgenera studied: *Quercus* and *Sclerophyllodrys*.

In the deciduous species of cool areas the trichomes appear only on the abaxial surface of the blade, while those of the evergreen species of drier areas appear on both leaf surfaces (Table 1; p. 122).

Radiate type hairs are found only in two species (*Q. broteroi*, and *Q. rotundifolia*), belonging to two different subgenera: *Quercus* and *Sclerophyllodrys*. This type is common in *Quercus* species (Hardin, 1976, 1979; Jones, 1989, Llamas et al., 1995), however in our study they have not appeared frequently.

The most frequently found hairs are fasciculate types (radiated fasciculate; stipitate fasciculate; parallel fasciculate) with the simple, uniseriate and not uniseriate, appearing in all studied species (Figure 3 and Table 1; p. 122).

It is necessary to highlight the presence of uniseriate-branched smooth hairs in *Q. estremadurensis* O. Schwarz (Figure 4; p. 122). This type of hair had not been detected to date in species of the Mediterranean, although they have been recorded in some species from North America such as *Q. palmeri* Engelm. (Hardin, 1976; Jones, 1986). The uniseriate, simple and branched hairs are included in glandular hair types (Jones, 1986), or secretory hair types by Camus (1936-54).

The results analysis shows that trichome type allows the separation of some species as shown by previous authors (Llamas et al., 1995; Penas et al., 1994; Thomson and Mohlenbrock, 1979).

Figure 5/ *Quercus rotundifolia* with crested, rounded stomata and verrucate stomata (black open point) (bar = 10 ηm).

3. Stomata

The stomata found can be divided into four types: a) smooth, elongated stomata; b) smooth, rounded stomata; c) crested, elongated stomata, and d) crested, rounded stomata. The rounded stomata appear mainly in evergreen species, subgenera *Sclerophyllodrys* and *Cerris*, while elongated stomata appear mainly in deciduous species of the subgenus *Quercus*. These results differ from those found by previous authors (Bussotti and Grossoni, 1997; Safou et al., 1988). In our study the ornamentation of the surface of the cells is not systematically related to phylogeny, but more to environmental parameters. In species such as *Q. estremadurensis*, *Q. canariensis* Willd., and *Q. faginea* subsp. *alpestris*, stomata have been found interchangeably with smooth and ornate (crested) guard cells attached. However, in evergreen species the stomata have ornate guard cells. The ornamentation degree of guard cells changes from slightly ornamented (with the stomata pore visible) to heavily ornamented (with the stomata pore not visible). Finally *Q. faginea* subsp. *alpestris*, has stomata with verrucate guard cells, similar to stomata of *Q. infectoria* Oliv., from Iran (Panahi et al., 2012) (Figure 5).

Study of floral and reproductive characters

1. Anthers

Anther morphology of the oaks of the SW Iberian Peninsula species segregate between the three subgenera studied: a) subgenus *Quercus* with rounded and smooth anthers of medium to large size; b) subgenus *Cerris* with apiculate and pilose anthers of medium to small size; and subgenus *Sclerophyllodrys* with apiculate and smooth anthers of medium to large size.

For hybrid taxa, anther studies have revealed anthers intermediate between the parents. Anthers of *Q.* ×*pacensis* F.M. Vázquez (= *Q. broteroi* × *Q. suber*), are rounded, of medium size and pilose (Figure 6). These results confirm that anther morphology can be used as a discriminating character between subgenera and some taxa.

2. Pollen

The results allow us to differentiate with clarity different groups of the genus *Quercus* in the SW Iberian Peninsula. If we look at the size of the pollen grains there are at least two major groups: a) species with pollen grains of up to 25 ηm, represented by species of the subgenus *Sclerophyllodrys* and b) taxa with pollen grains of more than 28 ηm, of the subgenera *Cerris* and *Quercus*. In addition, we can distinguish in the first group the species *Q. rotundifolia* and *Q. coccifera* L. by the pollen surface ornamentation. In *Q. rotundifolia* there is a surface with jagged ridges, and in *Q. coccifera*, a finely verrucate-jagged surface. In the case of the second group (b) pollen of species of the subgenus *Quercus* has a strongly verrucate surface, and that of the representative of the subgenus *Cerris* has a weakly verrucate pollen grain surface. (Figures 7 and 8).

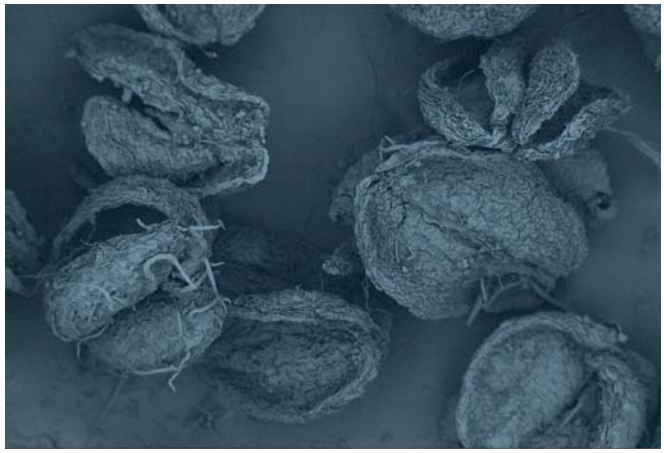

Figure 6/ *Quercus* ×*pacensis* with pilose and rounded anther (bar = 200 ηm).

These results were partially shown by Suárez (1987), Ruiz del Castillo (1988) and Denk and Grimm (2009); however these authors did not show the same results for *Q. broteroi* and *Q. faginea* subsp. *alpestris*. Also, they did not show a relationship between the surface of the pollen grains and the phylogenetic origin: (a) grains of pollen from species of the *Sclerophyllodrys* group, that grow in dry places, have ridges, while those of the other groups (b), that grow in cooler, wetter places, are verrucate (Denk and Grimm, 2009). The surface of the pollen grains is shown as a phylogenetic character, which may be related in some areas to the species habitat.

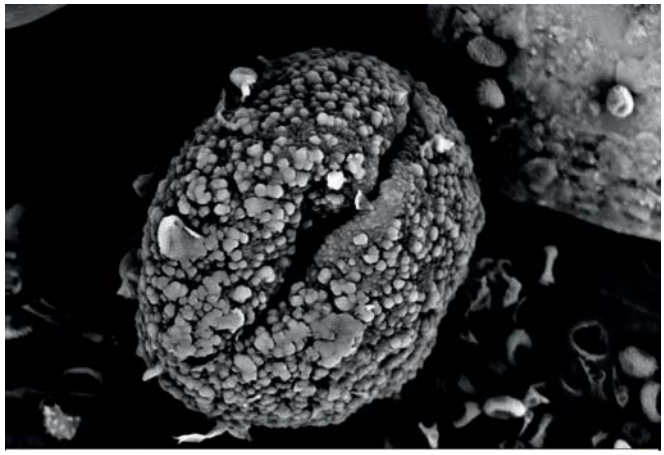

Figure 7/ Pollen grain surface of *Quercus faginea* subsp. *alpestris* (bar = 2 ηm).

Conclusion

These results highlight the foliage, floral and reproductive microcharacters that enable the segregation of *Quercus* species of the SW Iberian Peninsula. The characters studied that show the greatest diversity and expression are those associated with leaf morphology. Almost all species of the genus that have been studied can be differentiated by the foliar

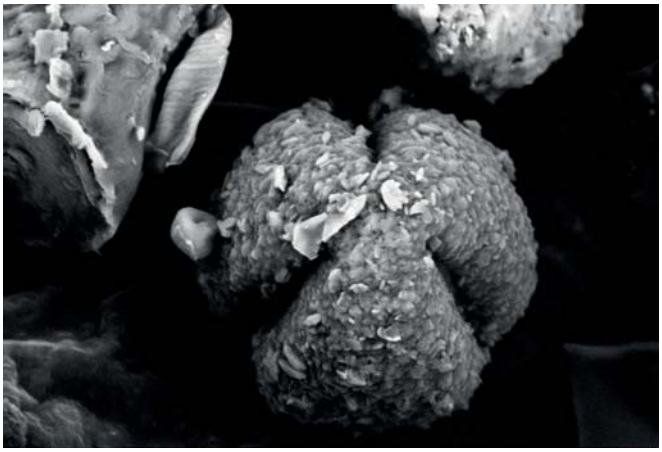

Figure 8/ Pollen grain surface of *Quercus rotundifolia* (bar = 3 ηm).

trichomes. These results have previously been expressed by Llamas et al., 1995 and Penas et al., 1994 from the north of the Iberian Peninsula; Hardin, 1976, 1979 and Thomson and Mohlenbrock 1979, from North America; and Panahi et al., 2012 from Iran.

The micromorphology of the pollen grain, the stomata, the anthers as well as leaf anatomy, represent characters that allow for the segregation of the different groups studied (subgenera) of the genus *Quercu*s, as determined by previous authors: Panahi et al., 2012, stomata; Denk and Grimm, 2009, pollen grain; and Hanson, 1917, leaf anatomy.

This study noted that environmental conditions create significant variations in some microcharacters. The thick walls of the epidemical cells appear in arid areas, the stomata with crested guard cells occur in species from dry places and the presence of multi-stellate hairs is associated with such habitats.

Finally, we found some typical microcharacters of *Quercus* species, which had not been previously observed in oaks of the SW Iberian Peninsula, such as: verrucate stomata (*Q. faginea* subsp. *alpestris)* uniseriate-branched hairs (*Q. estremadurensis*) and apiculate and pilose anthers (*Q. suber*).

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