

QUANTITATIVE MICROMORPHOLOGICAL CHARACTERIZATION AS A TOOL FOR THE TAXONOMIC IDENTIFICATION OF PHYLOGENOMIC OAK LINEAGES

SADDAN MORALES-SALDAÑA^{1,2}, SUSANA VALENCIA-ÁVALOS², M. SOCORRO GONZÁLEZ-ELIZONDO³,
 GONZALO CONTRERAS-NEGRETÉ⁴, ANDREW L. HIPPE^{5,6}, AND ANTONIO GONZÁLEZ-RODRÍGUEZ^{1*}

¹Instituto de Investigaciones en Ecosistemas y Sustentabilidad, Universidad Nacional Autónoma de México, Morelia, Michoacán, Mexico.

²Herbario de la Facultad de Ciencias, Departamento de Biología Comparada, Universidad Nacional Autónoma de México, Coyoacán, Mexico City, Mexico.

³CIIDIR Unidad Durango, Instituto Politécnico Nacional, Durango, Mexico.

⁴Ciencias Agrogenómicas, Escuela Nacional de Estudios Superiores, Universidad Nacional Autónoma de México, León, Mexico.

⁵The Morton Arboretum, Lisle, IL, USA.

⁶The Field Museum, Chicago, IL, USA

*Author for correspondence (agrodri@cieco.unam.mx)

Abstract

Background: Foliar micromorphological analysis has proven recurrently to be a valuable tool for assessing relationships at different taxonomical levels in the *Quercus* genus. However, *Quercus* is characterized by complicated taxonomic patterns resulting from frequent convergent evolution of vegetative characters, low differentiation among closely related species, and hybridization. These problems may be compounded by purely qualitative approaches to documenting the micromorphological intraspecific and interspecific variation.

Question: Which combinations of micromorphological traits can distinguish between the different genomic lineages within the *Quercus laeta* complex?

Studied species: *Quercus laeta*

Study site and dates: Mexico

Methods: We used scanning electron microscopy to qualitatively and quantitatively analyze trichomes and foliar waxes in 70 individuals from six previously identified lineages within the *Quercus laeta* complex. Our goal was to assess the taxonomic value of these micromorphological traits and to provide morphological support for the potential species status of these lineages.

Results: Our results indicate that the presence or absence of stellate trichomes, the number of both glandular and non-glandular trichomes, the number of arms, and the arm length of trichomes are key features for discriminating among lineages.

Conclusions: Our findings provided useful information to correctly identify and discriminate among genomic lineages of the *Quercus laeta* complex by highlighting quantitative (continuous) differences, contrary to the mostly qualitative (categorical) differences that have historically been used to discriminate among Mexican oak species.

Keywords: North America, scanning electron microscopy, species discovery, trichome, morphology.

Resumen

Antecedentes: El análisis micromorfológico foliar ha demostrado ser una herramienta valiosa para evaluar las relaciones a diferentes niveles taxonómicos en el género *Quercus*. Sin embargo, *Quercus* se caracteriza por patrones taxonómicos complejos resultantes de la frecuente evolución convergente de caracteres vegetativos, la baja diferenciación entre especies estrechamente relacionadas y la hibridación. Estos problemas pueden verse agravados por enfoques puramente cualitativos para documentar la variación micromorfológica intraespecífica e interespecífica.

Pregunta: ¿Qué combinaciones de caracteres micromorfológicos pueden distinguir los diferentes linajes genómicos dentro del complejo *Quercus laeta*?

Especie estudiada: *Quercus laeta*

Sitio y fecha de estudio: México

Métodos. Utilizamos microscopía electrónica de barrido para analizar cualitativa y cuantitativamente los tricomas y las ceras foliares de 70 individuos pertenecientes a seis linajes previamente identificados dentro del complejo *Quercus laeta*. Nuestro objetivo fue evaluar el valor taxonómico de estos rasgos micromorfológicos y brindar evidencia morfológica que respalde la posible clasificación de estos linajes como especies.

Resultados: Nuestros resultados indican que la presencia o ausencia de tricomas estrellados, el número de tricomas glandulares y no glandulares, el número de brazos y la longitud de los brazos de los tricomas son características clave para discriminar entre linajes.

Conclusiones: Nuestros hallazgos proporcionan información útil para identificar y discriminar correctamente entre linajes genómicos del complejo *Quercus laeta* al resaltar las diferencias cuantitativas (continuas), contrariamente a las diferencias cualitativas (categóricas) que históricamente se han utilizado para discriminar entre especies de encinos mexicanos.

Palabras clave: Norteamérica, microscopía electrónica de barrido, descubrimiento de especies, tricomas.

This is an open access article distributed under the terms of the Creative Commons Attribution License CCBY-NC (4.0) international.

<https://creativecommons.org/licenses/by-nc/4.0/>



The considerable variability in foliar micromorphological characters present in flowering plants makes these traits ideal for research in fields such as taxonomy, ecology, physiology, and evolution (Adebawale *et al.* 2014, Deng *et al.* 2014, Stephan *et al.* 2018, Ayaz & Gu 2024). Particularly, micromorphological characters have been successfully employed to solve taxonomic problems across different plant lineages since the 20th century (Payne 1978, Stace 1984, Deng *et al.* 2014, Siadati *et al.* 2020, Ayaz & Gu 2024).

Throughout history, the interspecific delimitation of oaks (*Quercus* L.) has challenged botanists (Cannon & Petit 2020, Valencia-A. 2021). In these efforts, foliar micromorphological analysis has recurrently proven to be a valuable tool to analyze relationships at different taxonomical levels (Tschan & Denk 2012, Deng *et al.* 2013, 2014) and, particularly, the characterization of trichome morphological features has been one of the most significant tools for the delimitation of oak species (Vázquez 2006, Scareli-Santos *et al.* 2007, Tschan & Denk 2012). Dyal (1936) and Camus (1936-38) established a functional classification based on glandular and non-glandular trichomes. Subsequently, with the introduction of the scanning electron microscope (SEM), several studies have contributed to the classification of different and new types of trichomes (Hardin 1976, 1979, Stace 1984, Jones 1986, Vázquez 2006, Tschan & Denk 2012), as well as to the delimitation of Asian (Kim *et al.* 2011, Panahi *et al.* 2012, Deng *et al.* 2015, 2017), European (Bussotti & Grossoni 1997, Tschan & Denk 2012, Fortini *et al.* 2015), and North American oak species (Dyal 1936, Hardin 1979, Manos 1993, Scareli-Santos *et al.* 2007, 2013, De Luna-Bonilla *et al.* 2024).

For Mexican oaks, scanning electron microscopy (SEM) has been used to describe micromorphological foliar structures that allow the recognition of combinations of characters to identify species within problematic taxonomic groups (Scareli-Santos *et al.* 2007, 2013, López-Caamal *et al.* 2017, De Luna-Bonilla *et al.* 2024). Nevertheless, important taxonomic confusion still exists, especially within the white oaks, section *Quercus* (Valencia-A. 2004). Intergradation in macromorphological characters is particularly common in the white oaks, resulting in numerous poorly resolved species complexes (Valencia-A. 2021, De Luna-Bonilla *et al.* 2024, Morales-Saldaña *et al.* 2024). Therefore, the effort to establish clearer limits among taxa requires the inclusion of additional traits and complementary taxonomic approaches. An example of this situation is *Quercus laeta* Liebm. (see Morales-Saldaña *et al.* 2022, 2024), a taxon with a history of controversy (McVaugh 1974, Romero Rangel *et al.* 2002, Valencia-A. 2004, Hipp *et al.* 2018), and which has been considered one of the most polymorphic Mexican oak species (Valencia-A. 2004). Recently, it has been demonstrated that *Quercus laeta* is not a highly polymorphic species but a complex of morphologically similar species that do not form a clade (Morales-Saldaña *et al.* 2022, 2024). This is supported by several phylogenetic studies, which show evidence that the *Q. laeta* species complex is polyphyletic. The complex comprises at least six lineages—potential species—each restricted geographically and ecologically (Hipp *et al.* 2018, 2020, Morales-Saldaña *et al.* 2024). Although these previous studies have demonstrated the existence of distinct phylogenetic entities, these lineages have not been thoroughly characterized morphologically, so a detailed analysis of micromorphological differentiation is required so their taxonomic status can be better understood.

In this context, we propose that micromorphological traits may support the genomic lineages proposed by Morales-Saldaña *et al.* (2024). From a morphological perspective, these traits will provide evidence that these lineages might be considered potentially different species. Here, we present a detailed quantitative study of trichome morphology and epicuticular waxes across the lineages of the *Quercus laeta* complex. The specific objectives of the study are: 1) to assess qualitative and quantitative trichome variability among lineages of the *Q. laeta* complex using scanning electron microscopy and 2) to determine the most useful combination of micromorphological characters for the identification of lineages within the *Q. laeta* complex. Moreover, we present this work as a case study demonstrating the potential of integrative taxonomy, an approach that combines multiple lines of evidence to delimit species and test taxonomic hypotheses, for disentangling the species complexes of Mexican oaks. Following this framework, molecular data first identify distinct lineages, which are then subjected to morphological diagnosis.

Materials and methods

Plant material and scanning electron microscopy. Leaf samples were collected from natural populations throughout most of the geographical range of the *Q. laeta* complex, representing a variety of distinct climatic regions (Figure 1). We analyzed 70 individuals and one leaf per individual representing all six genomic lineages identified by Morales-Saldaña *et al.* (2024). Fourteen samples corresponded to the centralis TMVBe (Trans-Mexican Volcanic Belt eastern) lineage, four to centralis TMVBc (Trans-Mexican Volcanic Belt central), 14 to laeta SMS (Sierra Madre del Sur), seven to laeta SMOc (Sierra Madre Occidental), 13 to prinopsis, and 18 to the previously called transmontana lineage, but here henceforth referred to as *Quercus* ‘skinny leaves’ to avoid confusion with *Quercus transmontana sensu* Trelease, and referring to its characteristically elongated leaves (Table S1). In all cases, mature leaves with no visible damage were collected during the fruiting period (August–November). After collection, leaves were dried at room temperature and conserved in plastic bags. The voucher specimens were deposited at MEXU and FCME herbaria. For SEM, we selected a mature leaf with no apparent damage from each individual. The middle region of the leaves was cut between the midrib and the margin into pieces of approximately 1 cm². Subsequently, the pieces were fixed on metal stubs using double-sided adhesive without further treatment. Micromorphological features were observed on the abaxial surface, and SEM micrographs were taken using a JEOL JSM-IT300 electron microscope (Microscopy Laboratory ENES UNAM Morelia).

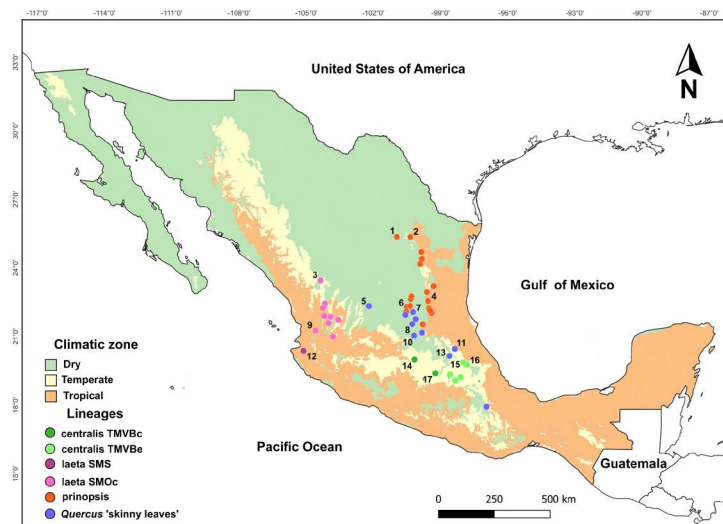


Figure 1. Distribution and localities sampled for the different lineages within the *Quercus laeta* complex. Each number corresponds to the population ID from Table S1.

Description of trichomes and wax types. A brief description was made of the different trichome types observed on the abaxial surface using the terminology and classification of Hardin (1976, 1979). The nomenclature proposed by Barthlott *et al.* (1998) was followed for the description of wax types.

Micromorphological quantitative comparison among species. To determine the trichome number on the abaxial leaf surface and obtain comparable sampling units, we divided the micrographs into grids of 0.05 mm². Subsequently, the length of trichome arms (μm), number of arms, and trichome number per unit area were analyzed on SEM images using the ImageJ software (Rasband 1997–2018). For compound trichomes (fasciculate and stellate), a maximum of eight arms per trichome was measured from their attachment point (excluding the pedestal in fasciculate trichomes) or the center of the central disc (in stellate trichomes) to the distal arm end. Also, we counted the number of arms for both fasciculate and stellate trichomes. For all variables, the minimum and maximum values were calculated, along

with the first and third quartiles of the data distribution. In addition, the mean and standard deviation were calculated, and the coefficients of variation were determined for the arm lengths of stellate and fasciculate trichomes. These calculations were performed using R v. 4.3.0 (R Core Team 2023). We counted the number of glandular and non-glandular trichomes in each grid. A total of 1993 trichome arms and 722 grids were analyzed.

To compare the distribution of the numeric variables among lineages, we visualized the data with violin graphs using the ggplot2 package in R. Violin graphs have the advantage of allowing visualization of the probability density of the data at different values by a kernel density estimator. Second, we carried out the Shapiro-Wilk goodness-of-fit test to evaluate the normality of all variables. Then, we conducted an analysis of variance (ANOVA) (no data needed to be transformed to achieve normality). Subsequently, a Tukey post hoc test was used to compare mean values of trichome number, number of arms, and arm length among the six *Q. laeta* complex lineages. These analyses were performed using R.

Multivariate analysis. We carried out a canonical discriminant analysis (CDA) to discriminate those lineages whose micromorphological differentiation was not clear and recognize the traits with higher contribution to the separation of lineages. We used the individual mean values of each trait evaluated (*i.e.*, length of trichome arms, number of trichome arms, and number of glandular and non-glandular trichomes). Only individuals that had metrics for all traits were included. Pillai's trace test was applied to determine the significance of the canonical axes from the CDA. The analysis was performed using the stats ggbiplot, MASS (Venables & Ripley 2002), and candisc (Friendly 2007, Friendly & Fox 2024) libraries in R. Finally, a taxonomic key was proposed for lineage identification based on all the qualitative and quantitative traits evaluated.

Results

Qualitative description of trichome and wax types. We identified three different trichome types: simple uniseriate (glandular), fasciculate and stellate (non-glandular), in the six lineages of the *Q. laeta* complex (Table 1; Figure 2A-E). We observed three different wax types among the samples (crystalloids, wax crusts, and wax layers) (Table 1; Figure S1) *sensu* Barthlott *et al.* (1998).

When observed fresh, the simple uniseriate trichomes consist of a single column of two or more thin-walled cells (Figure 2A, B, D, E). These cells are turgid in the juvenile state, but in herbarium specimens, they are usually collapsed due to loss of cellular content. This trichome type is found rarely on the abaxial leaf surfaces of the prinopsis and laeta SMS lineages, whereas it is common on the abaxial leaf surfaces of the lineages centralis TMVBc, centralis TMVBe, laeta SMOc, and *Quercus* 'skinny leaves'. The non-glandular trichomes are generally thick-walled, multicellular, and compound. Within this category, we can find fasciculate and stellate trichomes (Figure 2A, C, D, E, F). Fasciculate trichomes are arranged in tufts of (2) 6 to 8 (12) arms that originate from a common point. Fasciculate tufts are sessile with arms nearly smooth, slightly curled down and then twisted, the longer ones often intertwined. Individual arms are acicular or subulate, groups of arms or all arms fused basally, distally splaying out like a bouquet of flowers. Fasciculate trichomes occur on the abaxial surface of all investigated lineages (Table 1). This trichome type occurs both on veins and in intercostal areas. The stellate trichomes are multicellular, compound trichomes and usually persistent with (4) 7 - 9 (16) subulate to acicular arms radiating from a common origin appressed to the epidermis, hair base absent, but attachment point slightly rising above the surrounding epidermis. Stellate trichomes occur on the abaxial side of the leaf in centralis TMVBe, laeta SMS, and prinopsis lineages (Table 1). They are found predominantly in the intercostal regions, only occasionally on the veins. In the prinopsis lineage, the stellate trichomes are usually hidden below the fasciculate trichomes (Figure 2E), whereas in centralis TMVBe and laeta SMS lineages are not hidden below the fasciculate trichomes (Figure 2B, C). For wax types, the crust type was observed on fasciculate trichomes of all species (Figure S1A-B). Crystalloid waxes were observed exclusively on the abaxial surfaces of *Quercus* 'skinny leaves' and the laeta SMS lineage (Table 1; Figure S1C-D). Wax layers, which are defined as continuous coverings usually less than 1 μm thick without a prominent surface sculpture were also observed on the abaxial surface of all lineages (Figure S1E-F).

Table 1. Details of surface abaxial and trichome morphological characteristics in the six lineages of the *Quercus laeta* complex.

Lineages	Density	Surface	Wax types			Glandular trichomes	Non-glandular trichomes	
			Crystalloids	Wax crusts	Wax layer	Simple uniseriate	Fasciculate	Stellate
centralis TMVBc	Dense	Papillate	-	+	+	+	+	-
centralis TMVBe	Dense	Papillate	-	+	+	+	+	+
laeta SMS	Dense	Papillate	+	+	+	+	+	+
laeta SMOc	Sparse	Papillate	-	+	+	+	+	-
prinopsis	Very dense	Papillate	-	+	+	+	+	+
<i>Quercus</i> ‘skinny leaves’	Sparse	Papillate	+	+	+	+	+	-

Quantitative morphological comparison. A summary of quantitative leaf epidermal micromorphological features for all lineages is presented in [Table 2](#). The arm length of fasciculate and stellate trichomes differed, being longer in fasciculate than in stellate trichomes ([Figure 3A](#)). The prinopsis lineage showed the largest variability for the arm length of stellate trichomes (ALST), with arms ranging from 55 to 267 μm and a coefficient of variation (CV) of 32.69 % ([Figure 3B](#); [Table 2](#)). The ANOVA and the Tukey post hoc test revealed significant differences ($F_{2,987} = 31.11$; $P < 0.005$) in the ALST between laeta SMS and centralis TMVBe as well as between laeta SMS and prinopsis, but not between centralis TMVBe and prinopsis ([Figure 3B](#); [Table 3](#)).

Violin plots indicate great dispersion in the arm length of fasciculate trichomes (ALFT) ([Figure 3C](#)). The centralis TMVBe and laeta SMS lineages show smaller values with a mean of 242 and 203 μm , respectively ([Table 2](#); [Figure 3C](#)), whereas *Quercus* ‘skinny leaves’ showed the largest value, with arms ranging in length between 252 and 397 μm ([Table 2](#); [Figure 3C](#)). The ANOVA test revealed significant differences ($F_{5,997} = 52.82$; $P < 0.00001$) among lineages and the Tukey post hoc test indicated significant differences ($P < 0.05$) in almost all pairwise comparisons, with exception of centralis TMVBe – laeta SMOc, centralis TMVBe – prinopsis, laeta SMOc – prinopsis and prinopsis – *Quercus* ‘skinny leaves’ ([Table 3](#)).

Stellate trichomes have more arms on average (≥ 8) than fasciculate trichomes do (6 - 8) ([Figure 4A](#)). In the laeta SMS lineage, stellate trichomes can have up to 16 arms but are more likely to have between 8 and 11 arms ([Figure 4B](#)). Both centralis TMVBe and prinopsis lineages had a high frequency of stellate trichomes with 7 and 8 arms, though the prinopsis lineage had stellate trichomes with up to 11 arms ([Table 2](#); [Figure 4B](#)). The ANOVA test revealed significant differences ($F_{2,468} = 45.27$; $P < 0.00001$) in the number of arms of stellate trichomes among lineages and the Tukey post hoc test revealed significant differences ($P < 0.005$) between centralis TMVBe – laeta SMS and laeta SMS – prinopsis lineages, but not between centralis TMVBe – prinopsis ($P > 0.50$) ([Table 4](#)). For fasciculate trichomes, the laeta SMS lineage showed a greater number of arms with 8 or 9, although it can have up to 12 arms. In contrast, *Quercus* ‘skinny leaves’, and the centralis TMVBe and laeta SMOc lineages showed between 6 and 8 arms, whereas centralis TMVBe showed the smallest number of arms with 5 or 7 ([Table 2](#), [Figure 4C](#)). The ANOVA test revealed significant differences ($F_{5,718} = 34.46$; $P < 0.00001$) and the Tukey post hoc test showed significant differences ($P < 0.005$) in nine of the fifteen pairwise comparisons. The centralis TMVBe – centralis TMVBe, centralis TMVBe – laeta SMOc, centralis TMVBe – laeta SMOc, centralis TMVBe – prinopsis, centralis TMVBe – *Quercus* ‘skinny leaves’ and prinopsis – *Quercus* ‘skinny leaves’ comparisons were not significant ([Table 4](#)).

The lineages laeta SMS and prinopsis show a higher number of non-glandular trichomes ([Figure 5A](#)). The ANOVA test revealed significant differences ($F_{5,1068} = 86.79$; $P < 0.00001$) and the Tukey post hoc test revealed significant differences ($P < 0.005$) in non-glandular trichome number for almost all pairwise comparisons with the exception of centralis TMVBe – centralis TMVBe, centralis TMVBe – laeta SMOc, laeta SMOc – centralis TMVBe, *Quercus* ‘skinny leaves’ – centralis TMVBe, *Quercus* ‘skinny leaves’ – centralis TMVBe and *Quercus* ‘skinny leaves’ – laeta SMOc ([Table 5](#)). In contrast, centralis TMVBe and centralis TMVBe lineages show a higher number of glandular

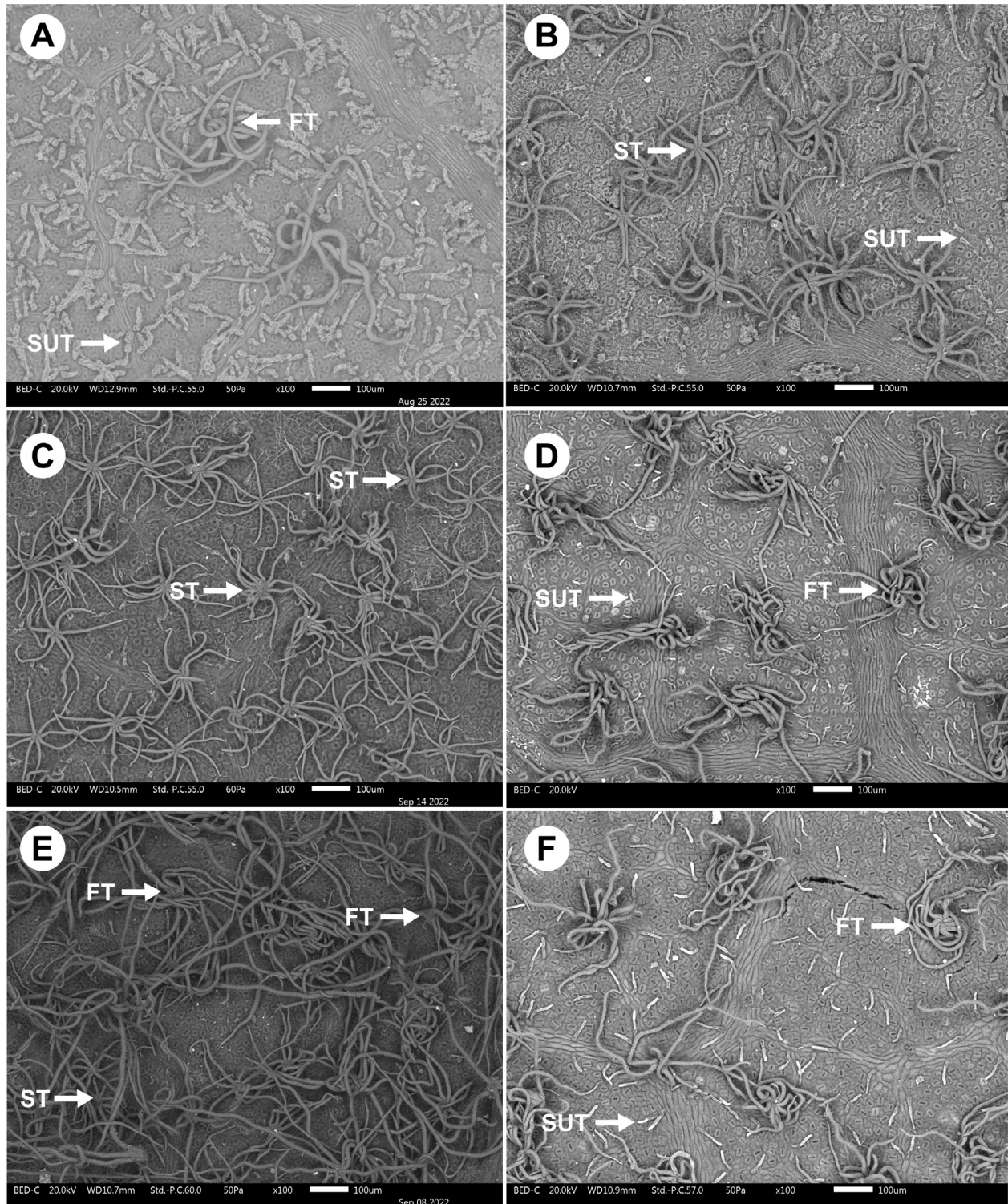


Figure 2. Scanning electron microscopy micrographs of trichome types from different *Quercus laeta* lineages. (A) Simple uniseriate (SUT) and fasciculate trichomes (FT) on centralis TMVBc. (B) Simple uniseriate (SUT) and stellate trichomes (ST) on centralis TMVBe. (C) Stellate trichomes (ST) on laeta SMS. (D) Simple uniseriate (SUT) and fasciculate trichomes (FT) on laeta SMS. (E) Fasciculate trichomes (FT) and stellate trichomes (ST) on prinopsis lineage. (F) Simple uniseriate (SUT) and fasciculate trichomes (FT) on *Q.* <skinny leaves>. Abbreviations: FT, fasciculate trichome; ST, stellate trichome; SUT, simple uniseriate trichome.

trichomes, although with a great spread of data (Figure 5B). In the case of the laeta SMS and prinopsis lineages, glandular trichome density was lower but with some outliers. The ANOVA test showed significant differences ($F_{5,1068} = 253.6$; $P < 0.0001$) and the Tukey post hoc test indicated significant differences ($P < 0.0001$) in glandular trichome number in all comparisons except for the pairwise comparison between laeta SMOc – *Quercus* ‘skinny leaves’ and laeta SMS – prinopsis (Table 5).

Table 2. Quantitative summary of leaf epidermal features on the abaxial surface of the lineages within *Quercus laeta* complex. NGT= number of non-glandular trichomes per sampled grid; NGT= Number of glandular trichomes per sampled grid; ALFT = Arm length of fasciculate trichomes; ALST = Arm length of stellate trichomes; ANST = Arm number for stellate trichomes; ANFT = Arm number for fasciculate trichomes; CV = coefficient of variation; NP = Not Present. The range of each variable corresponds to the first and third quartiles of the data distribution.

Lineages	NGT	NGT	ALFT (μm)	ALST (μm)	ANST	ANFT
centralis TMVBc	0 - 1 (2)	6 - 11 (18)	(89.43) 186.33 - 351.68 (509.85) (282.35 ± 105.22; CV = 37.26)	NP	NP	(4) 5 - 7 (8)
centralis TMVBe	0 - 1 (4)	4 - 8 (20)	(88.33) 187.44 - 283.73 (608.93) (242.61 ± 81.66; CV = 33.65)	(66) 108.26 - 157.36 (251.52) (132.73 ± 34.98; CV = 26.36)	(4) 7 - 8 (10)	(3) 6 - 8 (9)
laeta SMS	1 - 2 (9)	0 (4)	(96.95) 155.60 - 236.88 (488.89) (203.31 ± 65.07; CV = 32)	(50.02) 98.34 - 135.67 (239.12) (118.03 ± 29.29; CV = 24.82)	(6) 8 - 11 (16)	(6) 8 - 9 (12)
laeta SMOC	0 - 1 (5)	1 - 5 (13)	(164.9) 240.8 - 327.2 (460.1) (286.26 ± 62.92; CV = 21.98)	NP	NP	(3) 6 - 8 (9)
prinopsis	2 - 3 (6)	0 (3)	(82.61) 225.84 - 395.23 (538.36) (313.20 ± 111.96; CV = 35.74)	(55.01) 104.19 - 168.55 (267.65) (139.08 ± 45.47; CV = 32.69)	(4) 7 - 8 (11)	(4) 7 - 8 (10)
<i>Quercus</i> ‘skinny leaves’	0 - 1 (5)	1 - 5 (12)	(144) 252.7 - 397 (699.2) (338.44 ± 105.74; CV = 31.24)	NP	NP	(2) 6 - 8 (9)

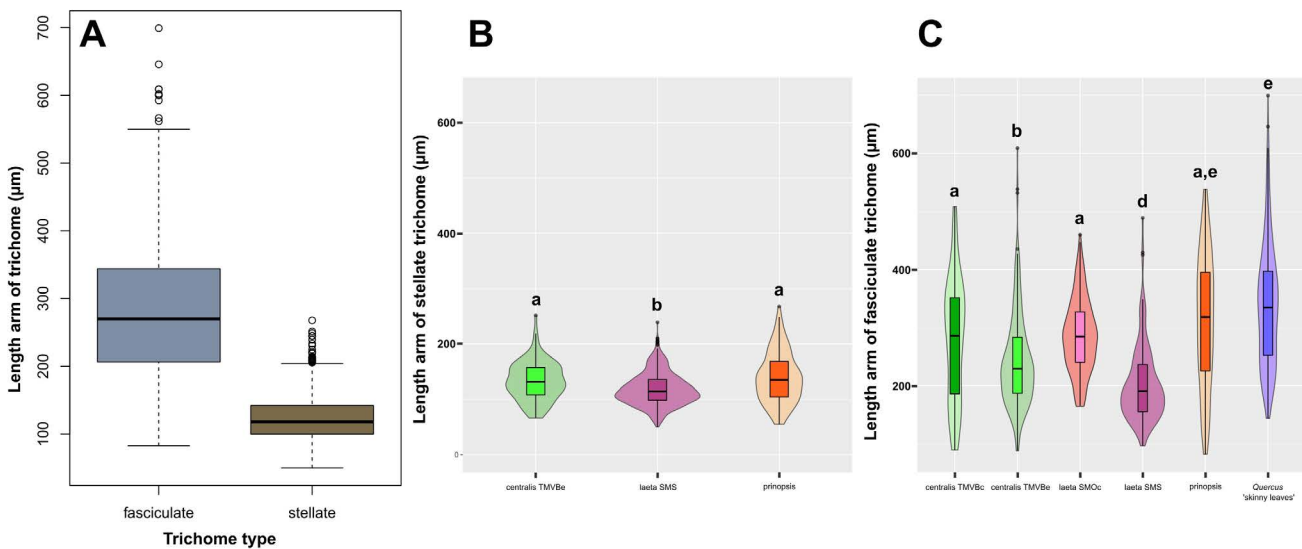
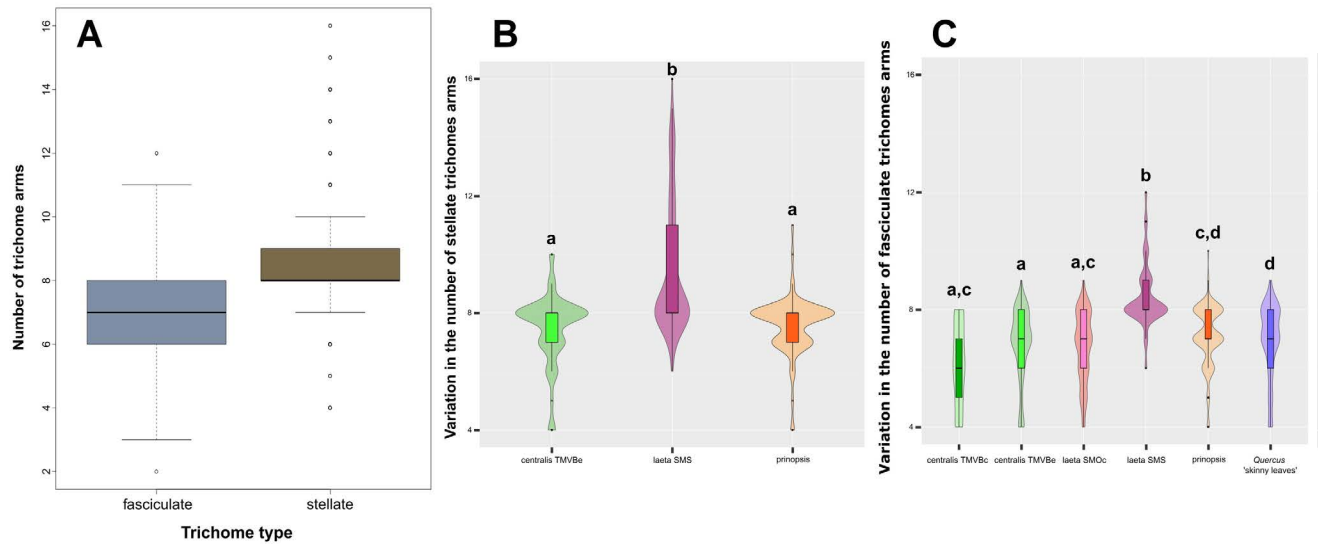


Figure 3. Length variation of trichome arms for each lineage and trichome type. A: Length variation of trichome arms between fasciculate and stellate trichomes; B: Length variation of stellate trichomes arms; C: Length variation of fasciculate trichomes arms. Wider sections of the violin plot represent a higher probability of observations taking a given value; the thinner sections correspond to a lower probability.

Table 3. Pairwise Tukey test among species for arm length of trichomes. *P*-values for the pairwise comparison among lineages for stellate trichomes (above), *P*-values for the pairwise comparison among lineages for fasciculate trichomes (below).

	centralis TMVBc	centralis TMVBe	laeta SMS	laeta SMOc	prinopsis	<i>Quercus</i> 'skinny leaves'
centralis TMVBc	-----	—	—	—	—	—
centralis TMVBe	0.045*	-----	0.000**	—	0.294	—
laeta SMS	0.000**	0.000**	-----	—	0.000**	—
laeta SMOc	0.999	0.000**	0.000**	-----	—	—
prinopsis	0.268	0.000**	0.000**	0.053	-----	—
<i>Quercus</i> 'skinny leaves'	0.000**	0.000**	0.000**	0.000**	0.086	-----

Significance codes: 0.001 '**' 0.05 '*', not significant ' '.

**Figure 4.** Arm number variation for each lineage and trichome type. A: Arm number variation between fasciculate and stellate trichomes; B: Arm number for stellate trichomes by lineage; C: Arm number for fasciculate trichomes by lineage. Wider sections of the violin plot represent a higher probability of observations taking a given value; the thinner sections correspond to a lower probability**Table 4.** Pairwise Tukey test among species for the number of arms of the trichomes. *P*-values for the pairwise comparison among lineages for stellate trichomes (above), *P*-values for the pairwise comparison among lineages for fasciculate trichomes (below).

	centralis TMVBc	centralis TMVBe	laeta SMS	laeta SMOc	prinopsis	<i>Quercus</i> 'skinny leaves'
centralis TMVBc	-----	—	—	—	—	—
centralis TMVBe	0.054	-----	0.000**	—	0.941	—
laeta SMS	0.000**	0.000**	-----	—	0.000**	—
laeta SMOc	0.890	0.114	0.000**	-----	—	—
prinopsis	0.000**	0.090	0.000***	0.000**	-----	—
<i>Quercus</i> 'skinny leaves'	0.002*	0.748	0.000***	0.001**	0.754	-----

Significance. codes: 0.001 '**' 0.05 '*', not significant ' '.

Table 5. Pairwise Tukey test among species for the density trichomes. *P*-values for the pairwise comparison among lineages for non-glandular trichomes (above), *P*-values for the pairwise comparison among lineages for glandular trichomes (below).

	centralis TMVBc	centralis TMVBe	laeta SMS	laeta SMOc	prinopsis	<i>Quercus</i> 'skinny leaves'
centralis TMVBc	-----	0.955	0.000**	0.973	0.000**	0.763
centralis TMVBe	0.000**	-----	0.000**	0.999	0.000**	0.978
laeta SMS	0.000**	0.000**	-----	0.000**	0.000**	0.000**
laeta SMOc	0.000**	0.000**	0.000 **	-----	0.000**	0.982
prinopsis	0.000**	0.000**	0.999	0.000**	-----	0.000**
<i>Quercus</i> 'skinny leaves'	0.000**	0.000**	0.000**	0.999	0.000**	-----

Significance. codes: 0.001 '**' 0.05 '*', not significant ' '.

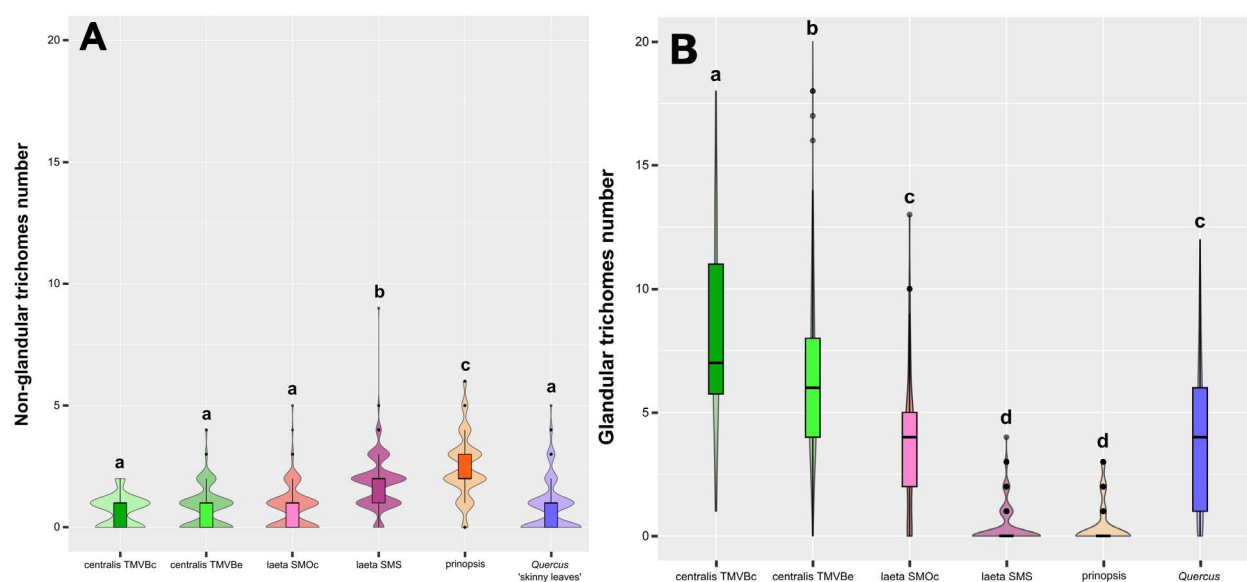


Figure 5. Trichome number variation among lineages. A: Number of non-glandular trichomes by unit area sampled; B: Number of glandular trichomes by unit area sampled. Wider sections of the violin plot represent a higher probability of observations taking a given value; the thinner sections correspond to a lower probability

Multivariate analysis. A canonical discriminant analysis was performed to discriminate lineages whose micromorphological differentiation remained ambiguous after pairwise quantitative comparisons (ANOVAs and Tukey's tests). The analysis of the evaluated traits identified two significant axes (Pillai's trace = 1.24, $P < 0.001$, 100 % of variation explained; Figure 6). The first axis of the CDA (61.9 % of variation) separated centralis TMVBe from the rest of the lineages, with the highest factor loadings for the number of glandular trichomes on the negative side of the axis. In contrast, the number of trichome arms and the number of non-glandular trichomes correlated positively with the second axis, contributing to the discrimination between laeta SMS and prinopsis lineages. The confusion matrix for the CDA showed a correct classification rate of 92 % for centralis TMVBe, whereas the correct classification rate was 100% for laeta SMS and 91 % for prinopsis (Table S2).

Discussion

The value of micromorphological characters, especially trichomes, for taxonomy and systematics across different plant groups has been emphasized by many authors (Gharemaninejada *et al.* 2012, Adebawale *et al.* 2014, Ali *et al.*

2020, Arabameri *et al.* 2020, Gissi *et al.* 2022). For the genus *Quercus*, nearly all authors consider characters of the indumentum to be important for species delimitation (Hardin 1976, 1979, Valencia Ávalos & Delgado Salinas 2003, Vázquez 2006, Tschan & Denk 2012, Scareli-Santos *et al.* 2013). However, *Quercus* is also characterized by showing complicated taxonomic patterns (Manos *et al.* 1999, Petit *et al.* 2004, Valencia-A. 2004, 2021, Morales-Saldaña *et al.* 2022) resulting from frequent parallel evolution of vegetative characters, pronounced intraspecific variation, low differentiation among closely related species, and hybridization (Tucker 1974, Baquedano *et al.* 2008, McVay *et al.* 2017, Kim *et al.* 2018, Crawl *et al.* 2020, Pérez-Pedraza *et al.* 2021, Valencia-A. 2021). Given this complexity, a purely qualitative assessment may not fully capture the range of intraspecific variation present. Therefore, a complementary quantitative approach can provide a robust framework for distinguishing morphologically similar entities and clarifying taxonomic boundaries (Vázquez 2006, Scareli-Santos *et al.* 2013). In this context, the present SEM study has provided useful information to characterize different genomic lineages and contribute to the recognition of these lineages as potentially independent species, by highlighting quantitative (continuous) rather than qualitative (categorical) differences, contrary to what has historically been used to discriminate among Mexican oak species (Liebmann 1854, McVaugh 1974, Romero Rangel *et al.* 2002).

Hardin's studies (1976, 1979) to characterize the variety of trichomes in North American oaks have been a pillar in the field of oak micromorphology, but this work has not translated obviously to the Mexican oaks. However, despite the effort to describe the micromorphology of Mexican oak species (Spellenberg & Bacon 1996, Valencia Ávalos & Delgado Salinas 2003, Vázquez 2006, Scareli-Santos *et al.* 2013), there have been few studies focused on evaluating inter- and intraspecific variation in a statistical framework (Scareli-Santos *et al.* 2007, López-Caamal *et al.* 2017, Sánchez-Acevedo *et al.* 2022). This lack of statistical investigation may be one cause of taxonomic difficulties in certain species complexes, as in the *Quercus laeta* complex. Our results indicate that although all lineages within the complex share several micromorphological traits, such as the presence of papillae, fasciculate, and glandular trichomes, as well as different types of wax on the abaxial surface (Table 1), certain micromorphological traits consistently discriminate lineages. In this context, the presence or absence of stellate trichomes as well as the number of both glandular and non-glandular trichomes should be considered key features. Notably, we observed a pattern of multiple bulges in the kernel density estimate of non-glandular trichomes. While this pattern could be influenced by sample size limitations, it may also reflect significant intra-lineage variation. Crucially, despite the known environmental plasticity of trichome density (Hernandez & Park 2022, Sánchez-Acevedo *et al.* 2022), our results revealed significant differentiation among lineages and, more importantly, a pattern of congruence between trichome micromorphology and phylogeny.

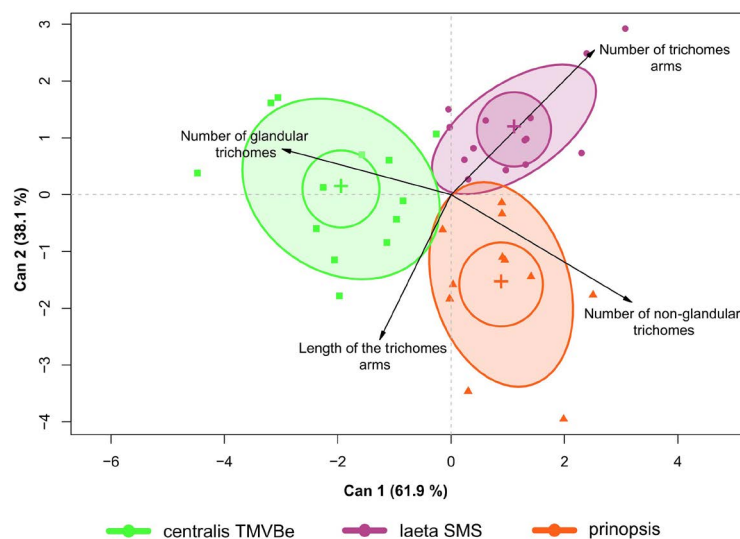


Figure 6. Scatterplot of scores derived from discriminant functions produced by discriminant analysis applied to micromorphological characters for three lineages of the *Quercus laeta* complex.

Since Liebmann (1854), the presence of stellate trichomes has only been discussed by Romero Rangel (2002) as one of the features to consider in the identification of *Q. laeta*, but specimens examined by her were collected in the Trans-Mexican Volcanic Belt, so they probably belonged to what we have recognized as lineages centralis TMVBc and centralis TMVBe (Morales-Saldaña *et al.* 2024). In contrast, other taxonomists, using samples from other geographic regions in Mexico, have not reported the presence of stellate trichomes in *Q. laeta* (McVaugh 1974, González-Villarreal 1986, Romero Rangel *et al.* 2014). According to our results, the absence of stellate trichomes on the abaxial leaf surface immediately discriminates *Quercus* ‘skinny leaves’, centralis TMVBc, and laeta SMOc lineages from the rest. Particularly, for *Quercus* ‘skinny leaves’, it was also possible to identify other distinctive traits, such as the presence of crystalloid waxes, as well as significantly longer arms of the fasciculate trichomes (up to 699 µm), and a lower density of non-glandular trichomes per area in comparison to laeta SMS and prinopsis lineages. This variation, together with the macromorphological morphometric differentiation presented by Morales-Saldaña *et al.* (2022), confirms that *Quercus* ‘skinny leaves’ is the most distinct lineage, supporting the hypothesis that it should be considered as an independent species (Morales-Saldaña *et al.* 2022, 2024).

Although glandular, stellate, and fasciculate trichomes were found in centralis TMVBe, laeta SMS, and prinopsis lineages, centralis TMVBe was characterized by an abaxial surface with a low number of non-glandular trichomes, but with a high density of simple uniseriate trichomes (glandular trichomes), distinguishing it from the laeta SMS and prinopsis lineages. Furthermore, arms of the fasciculate trichomes were significantly longer in centralis TMVBe than in laeta SMS but remarkably shorter than in the prinopsis lineage. For the discrimination of prinopsis and laeta SMS, the CDA analysis indicated that the number of non-glandular trichomes and the number of trichome arms are the two most important variables to differentiate between these lineages. In such a way, the prinopsis lineage is characterized by a high number of non-glandular trichomes as well as stellate and fasciculate trichomes with significantly longer arms than laeta SMS, which showed a higher arm number. Another difference is the presence of crystalloid waxes in laeta SMS, which are absent in the prinopsis lineage. Therefore, our results showed that quantitative differences are key to discriminating between these potential species as has been reported in other oak groups (Bussotti & Grossoni 1997). Although Tschan & Denk (2012) considered that quantitative data should be used with care for taxon delimitation, especially in cases of overlapping variability, as is the case in some lineages investigated here, the multiple samples representing different populations and the results of statistical tests suggest that quantitative micromorphology is capable of differentiating among genomic lineages and, therefore, could serve as a tool for species identification.

Our results enable us to interpret phenotypic variation as supporting the genomic and ecological evidence presented by Morales-Saldaña *et al.* (2024). This approach is crucial because overlooking phenotypic differentiation could reduce taxonomic entities to mere historical constructs with no biological relevance (Zapata & Jiménez 2012, Freudenstein *et al.* 2017). Within this context, our study emphasizes pairwise comparisons of quantitative characters among putative species (lineages), under the hypothesis that a bimodal (or polymodal) distribution of morphological variation indicates the presence of more than one species (Futuyma 1998, Zapata & Jiménez 2012).

Since both genetic and phenotypic discontinuities can result from geographic differentiation within a single species (Stephan *et al.* 2018, Maya-García *et al.* 2020), the description of new species based only on morphological variation could result in taxonomic inflation; therefore, an evolutionary framework is necessary to explain certain patterns found in this work. On the one hand, according to phylogenetic studies carried out by Hipp *et al.* (2018, 2020) and Morales-Saldaña *et al.* (2024), the *Q. laeta* complex is a non-monophyletic group, which suggests that the features they share, such as the presence of papillae, fasciculate and glandular trichomes as well as the presence of wax crusts and layers on the abaxial surface do not necessarily indicate close taxonomic affinity, and that they could be parallelisms (Tucker 1974, Struck *et al.* 2018). On the other hand, micromorphological differences found in this study, based on the presence of stellate trichomes and quantification of the length of the trichome arms, as well as the number of glandular and non-glandular trichomes and the number of trichome arms, provide a morphological identity that these evolutionarily independent lineages have acquired throughout the speciation process.

The use of SEM to qualitatively and quantitatively analyze micromorphological characteristics helps to correctly identify and discriminate among lineages of the *Quercus laeta* complex previously proposed by genomic data. Our study stresses the importance of stellate trichomes and trichome number as key features. In particular, the length and number of trichome arms are important taxonomic characters for identifying these lineages. Also, our data supports the recognition of these lineages as morphologically distinct, perhaps different species. In addition, we demonstrate that the characteristics proposed here to recognize the different species are consistent across multiple samples collected from the geographic range of each species. Trichome characters may be less plastic than leaf shape or habit, and less sensitive to environmental variation. Thus, our study can serve as a model for resolving ambiguities in other oak species complexes.

Taxonomic key based on leaf micromorphological characters to lineage identification.

- 1a. Abaxial surface with stellate trichomes altogether absent, fasciculate trichomes always present
- 2a. Abaxial surface with abundant glandular trichomes (approximately 6 - 18 per 0.05 mm²) centralis TMVBc
- 2b. Abaxial surface with a low number of glandular trichomes (0 - 5 per 0.05 mm²)
- 3a. Crystalloid waxes present, fasciculate trichomes arms 0.25 - 0.70 mm *Quercus* ‘skinny leaves’
- 3b. Crystalloid waxes absent, fasciculate trichomes arms 0.24 - 0.46 mm laeta SMOc
- 1b. Abaxial surface with stellate and fasciculate trichomes always present
- 4a. Abaxial surface densely pubescent, stellate trichomes hidden below the fasciculate trichomes, fasciculate trichomes arms notably long (0.23 - 0.40 mm) prinopsis
- 4b. Abaxial surface scarcely pubescent, stellate trichomes not hidden below the fasciculate trichomes, fasciculate trichomes arms notably short (0.16 - 0.24 mm)
- 5a. Abaxial surface with abundant glandular trichomes (approximately 4 - 8 per 0.05 mm²), stellate trichomes with 7 - 8 arms centralis TMVBe
- 5b. Abaxial surface generally without glandular trichomes (rarely up to 4 per 0.05 mm²), stellate trichomes with 8 - 16 arms laeta SMS

Supplementary material

Supplemental data for this article can be accessed here: <https://doi.org/10.17129/botsci.3879>

Acknowledgements.

We thank Oscar De Luna Bonilla, Ricardo Gaytán Legaria, and Maribel Arenas for their help with field collection. We thank Orlando Hernández Cristobal and Diana Luna Mañon for technical support with the SEM images.

Literature cited

- Adebowale A, Naidoo Y, Lamb J, Nicholas A. 2014. Comparative foliar epidermal micromorphology of Southern African *Strychnos* L. (Loganiaceae): taxonomic, ecological, and cytological considerations. *Plant Systematics and Evolution* **300**: 127-138. DOI: <https://doi.org/10.1007/s00606-013-0865-z>
- Ali M, Bahadur S, Hussain A, Saeed S, Khuram I, Ullah M, Shao WJ, Akhtar N. 2020. Foliar epidermal micromorphology and its taxonomic significance in *Polygonatum* (Asparagaceae) using scanning electron microscopy. *Microscopy Research and Technique* **83**: 1381-1390. DOI: <https://doi.org/10.1002/jemt.23529>
- Arabameri M, Khodayari H, Zarre S. 2020. Trichome micromorphology in *Alcea* L. and allied genera (Malvaceae) and its systematic implication. *Nordic Journal of Botany* **38**: 1-16. DOI: <https://doi.org/10.1111/njb.02540>
- Ayaz A, Gu Y. 2024. Macromorphological and foliar epidermal anatomical characteristics of *Lilium rosthornii* (Lili-

- aceae): Implications for morphological adaptations and taxonomic significance. *Microscopy Research and Technique* **87**: 2027-2033. DOI: <https://doi.org/10.1002/jemt.24577>
- Barthlott W, Neinhuis C, Cutler D, Ditsch F, Meusel I, Theisen I, Wilhelmi H. 1998. Classification and terminology of plant epicuticular waxes. *Botanical Journal of the Linnean Society* **126**: 237-260. DOI: <https://doi.org/10.1006/bojl.1997.0137>
- Baquadano FJ, Valladares F, Castillo FJ. 2008. Phenotypic plasticity blurs ecotypic divergence in the response of *Quercus coccifera* and *Pinus halepensis* to water stress. *European Journal of Forest Research* **127**: 495-506. DOI: <https://doi.org/10.1007/s10342-008-0232-8>
- Bussotti FP, Grossoni P. 1997. European and Mediterranean oaks (*Quercus* L.; Fagaceae): SEM characterization of the micromorphology of the abaxial leaf surface. *Botanical Journal of the Linnean Society* **124**: 183-199. DOI: <https://doi.org/10.1111/j.1095-8339.1997.tb01789.x>
- Camus A. 1936-1938. *Monographie du genre Quercus. Vol 1. Sous-genre Cyclobalanopsis. Sous-genre Euquercus, sections Cerris et Mesobalanus*. Academie des Sciences. Lechevalier, Paris.
- Cannon CH, Petit RJ. 2020. The oak syngameon: more than the sum of its parts. *New Phytologist* **226**: 978-983. DOI: <https://doi.org/10.1111/nph.16091>
- Crowl AA, Manos PS, McVay JD, Lemmon AR, Lemmon EM, Hipp AL. 2020. Uncovering the genomic signature of ancient introgression between white oak lineages (*Quercus*). *New Phytologist* **226**: 1158-1170. DOI: <https://doi.org/10.1111/nph.15842>
- De Luna-Bonilla OÁ, Valencia-Á S, Ibarra-Manríquez G, Morales-Saldaña S, Tovar-Sánchez E, González-Rodríguez A. 2024 Leaf morphometric analysis and potential distribution modelling contribute to taxonomic differentiation in the *Quercus microphylla* complex. *Journal of Plant Research* **137**: 3-19. DOI: <https://doi.org/10.1007/s10265-023-01495-z>
- Deng M, Hipp A, Song YG, Li QS, Coombes A, Cotton A. 2014. Leaf epidermal features of *Quercus* subgenus *Cyclobalanopsis* (Fagaceae) and their systematic significance. *Botanical Journal of the Linnean Society* **176**: 224-259. DOI: <https://doi.org/10.1111/boj.12207>
- Deng M, Li Q, Yang S, Liu Y, Xu J. 2013. Comparative morphology of leaf epidermis in the genus *Lithocarpus* and its implication in leaf epidermal feature evolution in Fagaceae. *Plant Systematics and Evolution* **299**: 659-681. DOI: <https://doi.org/10.1007/s00606-012-0751-0>
- Deng M, Song Y, Li QS, Coombes A, Li QJ. 2015. Clarification of identity of *Quercus patkoiensis* and *Q. semiseratoides* (Fagaceae) using leaf epidermal features. *Plant Systematics and Evolution* **301**: 849-860. DOI: <https://doi.org/10.1007/s00606-014-1119-4>
- Deng M, Xiang LX, Song GY, Coombes A, Yang RX, Xiong SY, Li SQ, 2017. Leaf epidermal features of *Quercus* group Ilex (Fagaceae) and their application to species identification. *Review of Palaeobotany and Palynology* **237**: 10-36. DOI: <https://doi.org/10.1016/j.revpalbo.2016.11.006>
- Dyal SA. 1936. A key to species of oaks of eastern North America based on foliage and twig characters. *Rhodora* **38**: 53-63
- Fortini P, Antonecchia G, Di Marzio P, Maiuro L, Viscosi V. 2015. Role of micromorphological leaf traits and molecular data in taxonomy of three sympatric white oak species and their hybrids (*Quercus* L.). *Plant Biosystems* **149**: 546-558. DOI: <https://doi.org/10.1080/11263504.2013.868374>
- Freudenstein JV, Broe MB, Folk RA, Sinn BT. 2017. Biodiversity and the species concept—lineages are not enough. *Systematic Biology* **66**: 644-656. DOI: <https://doi.org/10.1093/sysbio/syw098>
- Friendly M. 2007. HE plots for Multivariate General Linear Models. *Journal of Computational and Graphical Statistics* **16**: 421-444. DOI: <https://doi.org/10.1198/106186007X208407>
- Friendly M, Fox J. 2024. Candisc: Visualizing Generalized Canonical Discriminant and Canonical Correlation Analysis. R package version 0.9.0, <https://CRAN.R-project.org/package=heplots>.
- Futuyma DJ. 1998. *Evolutionary Biology*. Sunderland (MA): Sinauer.
- Gharemaninejada F, Khalili Z, Maassoumi AA, Mirzaie-Nodoushan H, Riahi M. 2012. Leaf epidermal features

- of *Salix* species (Salicaceae) and their systematic significance. *American Journal of Botany* **99**: 769-777. DOI: <https://doi.org/10.3732/ajb.1100019>
- Gissi DS, Seixas DP, Fortuna-Perez AP, Torke BM, Simon MF, Souza G, Lewis PG, Rodrigues TM. 2022. Leaf and stem anatomy of the *Stylosanthes guianensis* complex (Aubl.) Sw. (Leguminosae, Papilionoideae, Dalbergieae) and its systematic significance. *Flora* **287**: 151992. DOI: <https://doi.org/10.1016/j.flora.2021.151992>
- González-Villarreal LM. 1986. *Contribución al Conocimiento del Género Quercus (Fagaceae) en el Estado de Jalisco*. Guadalajara, México: Instituto de Botánica, Colección Flora de Jalisco, Universidad de Guadalajara.
- Hardin JW. 1976. Terminology and classification of *Quercus* trichomes. *Journal of the Elisha Mitchell Scientific Society* **92**: 151-161.
- Hardin JW. 1979. Patterns of variation in foliar trichomes of eastern North American *Quercus*. *American Journal of Botany* **66**: 576-585. DOI: <https://doi.org/10.1002/j.1537-2197.1979.tb06260.x>
- Hernandez JO, Park BB. 2022. The leaf trichome, venation, and mesophyll structural traits play important roles in the physiological responses of oak seedlings to water-deficit stress. *International Journal of Molecular Sciences* **23**: 8640. DOI: <https://doi.org/10.3390/ijms23158640>
- Hipp AL, Manos PS, González-Rodríguez A, Hahn M, Kaproth M, McVay JD, Avalos SV, Cavender-Bares J. 2018. Sympatric parallel diversification of major oak clades in the Americas and the origins of Mexican species diversity. *New Phytologist* **217**: 439-452. DOI: <https://doi.org/10.1111/nph.14773>
- Hipp AL, Manos PS, Hahn M, Avishai M, Bodénes C, Cavender-Bares J, Cowl A, Deng M, Denk T, Fitz-Gibbon S, Gailing O, González-Elizondo MS, González-Rodríguez A, Grimm GW, Jiang XL, Kremer A, Lesur I, McVay JD, Plomion C, Rodríguez-Correa H, Schulze ED, Simeone MC, Sork VL, Valencia-Avalos S. 2020. Genomic landscape of the global oak phylogeny. *New Phytologist* **226**: 1-15. DOI: <https://doi.org/10.1111/nph.16162>
- Jones JH. 1986. Evolution of the Fagaceae: the implications of foliar features. *Annals of the Missouri Botanical Garden* **73**: 228-275. DOI: <https://doi.org/10.2307/2399112>
- Kim WK, Cho HD, Kim PG. 2011. Morphology of Foliar Trichomes of the Chinese Cork Oak *Quercus variabilis* by Electron Microscopy and Three-Dimensional Surface Profiling. *Microscopy and Microanalysis* **17**: 461-468. DOI: <https://doi.org/10.1017/S1431927611000407>
- Kim BY, Wei X, Fitz-Gibbon S, Lohmueller KE, Ortego J, Gugger PF, Sork VL. 2018. RADseq data reveal ancient, but not pervasive, introgression between Californian tree and scrub oak species (*Quercus* sect. *Quercus*: Fagaceae). *Molecular Ecology* **27**: 4556-4571. DOI: <https://doi.org/10.1111/mec.14869>
- Liebmann FM. 1854. *Oversigt over det kongelige danske videnskabernes selskabs forhandlinger og dets medlemmers arbejder*. *Bulletin de l'Académie royale des sciences et des lettres de Danemark*: 179.
- López-Caamal A, Ruiz-Amaro LDC, Zepeda-Rodríguez A, Mussali-Galante P, Tovar-Sánchez E. 2017. Micromorphological character expression of the hybrid *Quercus* × *dysophylla* and its parental species (*Q. crassifolia* and *Q. crassipes*). *Botanical Sciences* **95**: 375-389. DOI: <https://doi.org/10.17129/botsci.875>
- Manos PS. 1993. Foliar trichome variation in *Quercus* section *Protobalanus* (Fagaceae). *Sida* **15**: 391-403.
- Manos PS, Doyle JJ, Nixon KC. 1999. Phylogeny, biogeography, and processes of molecular differentiation in *Quercus* subgenus *Quercus* (Fagaceae). *Molecular Phylogenetics and Evolution* **12**: 333-349. DOI: <https://doi.org/10.1006/mpev.1999.0614>
- Maya-García R, Torres-Miranda A, Cuevas-Reyes P, Oyama K. 2020. Morphological differentiation among populations of *Quercus elliptica* Née (Fagaceae) along an environmental gradient in Mexico and Central America. *Botanical Sciences* **98**: 50-66. DOI: <https://doi.org/10.17129/botsci.2395>
- McVaugh R. 1974. Fagaceae. *Flora Novo-galiciana*. Ann Arbor: University of Michigan Herbarium.
- McVay JD, Hauser D, Hipp AL, Manos PS. 2017. Phylogenomics reveals a complex evolutionary history of lobed-leaf white oaks in western North America. *Genome* **60**: 733-742. DOI: <https://doi.org/10.1139/gen-2016-0206>
- Morales-Saldaña S, Valencia-Ávalos S, Oyama K, Tovar-Sánchez E, Hipp AL, González-Rodríguez A. 2022. Even more oak species in Mexico? Genetic structure and morphological differentiation support the presence of at least

- two specific entities within *Quercus laeta*. *Journal of Systematics and Evolution* **60**: 1124-1139. DOI: <https://doi.org/10.1111/jse.12818>
- Morales-Saldaña S, Hipp AL, Valencia-Ávalos S, Hahn M, González-Elizondo MS, Gernandt SD, Pham KK, Oyama K, González-Rodríguez A. 2024. Divergence and reticulation in the Mexican white oaks: ecological and phylogenomic evidence on species limits and phylogenetic networks in the *Quercus laeta* complex (Fagaceae). *Annals of Botany* **133**: 1007-1024. DOI: <https://doi.org/10.1093/aob/mcae030>
- Panahi P, Jamzad Z, Pourmajidian MR, Fallah A, Pourhashemi M. 2012. Foliar epidermis morphology in *Quercus* (subgenus *Quercus*, section *Quercus*) in Iran. *Acta Botanica Croatica* **71**: 95-113. DOI: <https://doi.org/10.2478/v10184-010-0029-y>
- Payne WW. 1978. A glossary of plant hair terminology. *Brittonia* **30**: 239-255. DOI: <https://doi.org/10.2307/2806659>
- Pérez-Pedraza A, Rodríguez-Correa H, Valencia-Ávalos S, Torres-Miranda CA, Arenas-Navarro M, Oyama K. 2021. Effect of hybridization on the morphological differentiation of the red oaks *Quercus acutifolia* and *Quercus grahamii* (Fagaceae). *Plant Systematics and Evolution* **307**: 37. <https://doi.org/10.1007/s00606-021-01757-0>
- Petit RJ, Bodénès C, Ducouso A, Roussel G, Kremer A. 2004. Hybridization as a mechanism of invasion in oaks. *New Phytologist* **161**: 151-164. DOI: <https://doi.org/10.1046/j.1469-8137.2003.00944.x>
- R Core Team 2023. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Rasband WS. 1997-2018. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <https://imagej.net/ij/> (accessed 14 May, 2024).
- Romero Rangel SR, Carlos E, Zenteno R, de Lourdes M, Enríquez A. 2002. El género *Quercus* (Fagaceae) en el estado de México. *Annals of the Missouri Botanical Garden* **89**: 551-593. DOI: <https://doi.org/10.2307/3298595>
- Romero Rangel SR, Rojas ZCE, Rubio LEL. 2014. Familia Fagaceae. *Flora del Bajío y regiones adyacentes*. **181**: 1-167.
- Sánchez-Acevedo V, Torres-Miranda A, Gutiérrez-Arellano C, Hernández-Esquivel KB, Oyama K. 2022. Variability in leaf morphological traits of an endemic Mexican oak (*Quercus mexicana* Bonpl.) along an environmental gradient. *Botanical Sciences* **100**: 579-599. DOI: <https://doi.org/10.17129/botsci.3001>
- Scareli-Santos C, Herrera-Arroyo ML, Sánchez-Mondragón ML, González-Rodríguez A, Bacon J, Oyama K. 2007. Comparative analysis of micromorphological characters in two distantly related Mexican oaks, *Quercus conzattii* and *Q. eduardii* (Fagaceae), and their hybrids. *Brittonia* **59**: 37-48. DOI: [https://doi.org/10.1663/0007-196X\(2007\)59\[37:CAOMCI\]2.0.CO;2](https://doi.org/10.1663/0007-196X(2007)59[37:CAOMCI]2.0.CO;2)
- Scareli-Santos C, Sánchez-Mondragón ML, González-Rodríguez A, Oyama K. 2013. Foliar micromorphology of Mexican oaks (*Quercus*: Fagaceae). *Acta Botanica Mexicana* **104**: 31-52. DOI: <https://doi.org/10.21829/abm104.2013.56>
- Siadati S, Salmaki, Y, Bräuchler C. 2020. Trichome morphology provides phylogenetically informative signal for generic delimitation in tribe Marrubieae (Lamiaceae). *Flora* **273**: 151720. DOI: <https://doi.org/10.1016/j.flora.2020.151720>
- Spellenberg R, Bacon JR. 1996. Taxonomy and distribution of a natural group of black oaks of Mexico (*Quercus*, section Lobatae, subsection Racemiflorae). *Systematic Botany* **21**: 85-99. DOI: <https://doi.org/10.2307/2419565>
- Stace CA. 1984. *The taxonomic importance of the leaf surface*. In: Heywood VH, Moore DH. eds. *Current Concepts in Plant Taxonomy*. London: Academic Press.
- Stephan JM, Teeny PW, Vessella F, Schirone B. 2018. Oak morphological traits: between taxa and environmental variability. *Flora* **243**: 32-44. DOI: <https://doi.org/10.1016/j.flora.2018.04.001>
- Struck TH, Feder JL, Bendiksby M, Birkeland S, Cerca J, Gusarov VI, Kistenich S, Larsson HK, Liow HL, Nowak DM, Stedje B, Bachmann L, Dimitrov D. 2018. Finding evolutionary processes hidden in cryptic species. *Trends in Ecology and Evolution* **33**: 153-163. DOI: <https://doi.org/10.1016/j.tree.2017.11.007>
- Tschan FG, Denk T. 2012. Trichome types, foliar indumentum and epicuticular wax in the Mediterranean gall oaks,

- Quercus* subsection Galliferae (Fagaceae): implications for taxonomy, ecology, and evolution. *Botanical Journal of the Linnean Society* **169**: 611-644. DOI: <https://doi.org/10.1111/j.1095-8339.2012.01233.x>
- Tucker JM. 1974. Patterns of parallel evolution of leaf form in new world oaks. *Taxon* **23**: 129-154. DOI: <https://doi.org/10.2307/1218095>
- Valencia-A S. 2004. Diversity of the genus *Quercus* (Fagaceae) in Mexico. *Botanical Sciences* **75**: 33-53. DOI: <https://doi.org/10.17129/botsoci.1692>
- Valencia-A S. 2021. Species delimitation in the genus *Quercus* (Fagaceae). *Botanical Sciences* **99**: 1-12. DOI: <https://doi.org/10.17129/botsoci.2658>
- Valencia Ávalos S, Delgado Salinas A. 2003. Los tricomas foliares en la caracterización de un grupo de especies del género *Quercus*, sección *Lobatae* (Fagaceae). *Anales del Instituto de Biología de la Universidad Nacional Autónoma de México Serie Botánica* **74**: 5-15.
- Vázquez ML. 2006. Trichome morphology in selected mexican red oaks species (*Quercus* section *Lobatae*). *Sida* **22**: 1091-1110
- Venables WN, Ripley BD. 2002. *Modern Applied Statistics with S*, Fourth edition. Springer, New York.
- Zapata F, Jiménez I. 2012. Species delimitation: inferring gaps in morphology across geography. *Systematic Biology* **61**: 179. DOI: <https://doi.org/10.1093/sysbio/syr084>

Associate editor: Silvia Aguilar Rodríguez

Author contributions: SMS: Writing original draft, Writing review and editing, Visualization, Methodology, Software, Investigation, Formal analysis, Data curation, Validation, Conceptualization. SVA: Writing review and editing, Investigation. MSGE: Writing review & editing, Investigation. GCN: Writing review and editing, Formal analysis, Investigation. ALH: Writing reviews and editing, Visualization, Investigation. AGR: Writing original draft, Writing review and editing, Resources, Funding acquisition, Project administration, Supervision, Investigation, Validation, Visualization, Conceptualization.

Supporting Agencies (alphabetical order): SMS thanks Posgrado en Ciencias Biológicas-Universidad Nacional Autónoma de México (UNAM) and Consejo Nacional de Ciencia y Tecnología (CONACYT) for the PhD scholarship and financial support to develop graduate studies at UNAM (scholarship/CVU: 483720/624511). The authors also acknowledge funding from the Dirección General de Asuntos del Personal Académico (DGAPA) through the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT IN210020). Furthermore, the authors would like to express their sincere gratitude to the National Geographic Society for their generous funding (grant NGS-73961R-20).

Conflict of interests: The authors declare that there is no conflict of interest, financial or personal, in the information, presentation of data, and results of this article.