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Leaf epidermis micromorphology of several species of *Ferula* and *Leutea* from Iran

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Abstract

Micromorphological features of the leaf epidermis and stomata were studied in selected species of *Ferula* and *Leutea* from Iran using light and scanning electron microscopy (LM and SEM). The aim was to assess the taxonomic value of these characters and to compare them with recent molecular findings regarding the affinities among these genera. Stomatal dimensions, density, and index were quantified and compared among species. *Ferula stenocarpa* showed the longest stomata and the highest stomatal index, while *F. ovina* had the widest stomata and *Leutea cupularis* showed the lowest index. The epidermal cells were mostly irregular or polygonal, with surface textures ranging from striate to smooth or wax-covered. Most species exhibited anisocytic stomata, while *F. gummosa* displayed anomocytic types. These micromorphological patterns support the close relationship between *Ferula* and *Leutea*, and provide evidence consistent with the reclassification of *Dorema* within *Ferula*. The results highlight the diagnostic value of epidermal micromorphology in delimiting species and understanding phylogenetic relationships within the Apiaceae.

Keywords: Apiaceae, anatomy, cuticle, stomata, taxonomy, Umbelliferae

ریزریخت‌شناسی اپیدرم برگ برخی از گونه‌های *Ferula* و *Leutea* در ایران

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خلاصه

ویژگی‌های ریزریخت‌شناسی اپیدرم برگ و روزنه‌ها در گونه‌های منتخب از جنس‌های *Ferula* و *Leutea* (کرفسیان) در ایران با استفاده از میکروسکوپ نوری (LM) و الکترونی روبشی (SEM) مورد مطالعه قرار گرفت. هدف این پژوهش ارزیابی ارزش تاکسونومیک این صفات و مقایسه آن‌ها با یافته‌های مولکولی اخیر در خصوص خویشاوندی این دو جنس بود. ابعاد، تراکم و شاخص روزنه‌ها اندازه‌گیری و میان گونه‌ها مقایسه گردید. در این میان، *F. stenocarpa* دارای بلندترین روزنه‌ها و بالاترین شاخص روزنه، *F. ovina* دارای پهن‌ترین روزنه‌ها و *L. cupularis* دارای پایین‌ترین شاخص روزنه بود. سلول‌های اپیدرمی عمدتا نامنظم یا چندضلعی و بافت سطحی آن‌ها از شیاردار تا صاف یا پوشیده از واکس (موم) متغیر بود. بیشتر گونه‌ها دارای روزنه‌های آنیزوسیتیک بودند، درحالی‌که *F. gummosa* روزنه‌های آنوموسیتیک را نشان داد. یافته‌های مطالعه حاضر، نشان‌دهنده نزدیکی خویشاوندی میان *Ferula* و *Leutea* است و بازطبقه‌بندی جنس *Dorema* در *Ferula* را تأیید می‌کند. نتایج این پژوهش، ارزش تشخیصی ریزریخت‌شناسی اپیدرم برگ را نیز در تفکیک گونه‌ها و درک روابط فیلوژنتیکی در کرفسیان برجسته می‌سازد.

واژه‌های کلیدی: آناتومی، تاکسونومی، چتریان، روزنه، کرفسیان، کوتیکول

Introduction

Apiaceae is among the largest families of flowering plants, comprising approximately 434 genera and nearly 3780 species and is divided into four subfamilies: Apioideae, Azorelloideae, Mackinlayoideae, and Saniculoideae (APG IV 2016, Plunkett *et al.* 2018). The family includes many essential vegetable crops and is widely distributed globally (Sayed-Ahmad *et al.* 2017). Iran is a significant center of diversification for Apiaceae, hosting more than 120 genera and around 360 species (Mozaffarian 2007, Amiri & Joharchi 2016), making it the third richest country in Apiaceae species diversity after China and Turkey.

The genus *Ferula* L., locally known as “Koma” in Persian, stands out with approximately 170 species distributed from Central Asia to Northern Africa, and with 32 species identified to date in Iran (Mozaffarian 2007, Amiri & Joharchi 2016, Yaqoob & Nawchoo 2016) of which, 20 species are endemic to Iran (Ghahremaninejad *et al.* 2025). *Ferula* is valued for its pharmacological properties, including antiseptic, antinociceptive, antispasmodic, digestive, carminative, and diuretic effects (Yaqoob & Nawchoo 2016). The subtribe Ferulinae comprises six genera and more than 200 species distributed from Western Europe to China (Panahi *et al.* 2015). Recent molecular studies showed that, the genera *Leutea* Pimenov, *Dorema* D.Don, and *Ferula* form part of this subtribe (Panahi *et al.* 2018, Puchałka *et al.* 2023).

The genus *Leutea* is distinguished by morphological features such as rigid cylindrical ultimate segments, and the presence of only hermaphroditic flowers that set it apart from *Ferula.* However, molecular studies have presented differing views on the taxonomic position of this genus. Kurzyna-Młynik *et al.* (2008) suggested *Leutea* to be grouped with *Ferula* members in the subtribe Ferulinae, whereas Panahi *et al.* (2015) and Puchałka *et al.* (2023) placed *Leutea* as a sister group to *Ferula* within the same subtribe. *Dorema* is characterized by morphological features such as simple umbels arranged in racemose inflorescences and short, equal-length pedicels forming globular umbels, features that are also common in several *Ferula* species (Mozaffarian 2007). Recent developmental and phylogenetic analyses have also highlighted floral and inflorescence similarities between *Ferula* and *Dorema*, supporting their close relationship within subtribe Ferulinae (Claßen-Bockhoff & Ajani 2025).

Although several molecular studies have addressed the relationships among *Ferula*, *Leutea*, and *Dorema*; their phylogenetic boundaries remain somewhat uncertain. The present study aims to provide additional micromorphological evidence that may contribute to clarifying these relationships within *Ferulinae* (Panahi *et al*. 2015, Puchałka *et al*. 2023).

The primary goal of this research was to utilize micromorphological features of the leaf epidermis to clarify the phylogenetic position of the genera *Leutea* and *Dorema* within the subtribe Ferulinae (tribe Scandiceae, subfamily Apioideae). Previous molecular and taxonomic studies (Downie *et al.* 2010) have addressed this subtribe, but its internal relationships remain unresolved. In the present study, an alternative micromorphological feature of the leaf epidermis is used to clarify the phylogenetic position of *Leutea* and confirm the placement of former *Dorema* within the subtribe Ferulinae. Recent molecular studies have provided inconsistent classifications within Ferulinae, especially regarding the generic limits of *Ferula*, *Leutea*, and *Dorema* (Kurzyna-Młynik *et al.* 2008, Panahi *et al.* 2015, Puchałka *et al.* 2023). Therefore, the present study aims to provide new micromorphological evidence to help resolve these taxonomic uncertainties. Micromorphological studies of the leaf epidermis have emerged as an important tool in taxonomic studies, providing significant evidence for refining taxonomy and phylogeny (Ghahremaninejad *et al.* 2012, Karbalaei *et al.* 2021).

Investigating the micromorphology of the epidermis and stomata can establish taxonomic relationships and resolve ambiguities within diverse plant groups (Ostroumova 1990, Ostroumova & Lavrova 1991, Ostroumova & Kljuykov 2007, Zhou & Liu 2018, Wang *et al.* 2022, Song *et al.* 2024). This approach is particularly relevant for *Ferula*, where morphological similarities between species pose notable taxonomic challenges. Additionally, previous studies (Ostroumova 1990, Ostroumova & Lavrova 1991, Ostroumova & Kljuykov 2007) have highlighted the taxonomic value of stomatal types and the number of surrounding cells in various genera and species groups within Apiaceae, emphasizing the importance of these micromorphological features. This study investigates the morphology and micromorphology of the epidermis and stomata in selected species of the genus *Ferula*, mostly from central and southern parts of Iran, including two species of *Leutea*, to examine micromorphological consistency following recent molecular studies. By identifying distinct micro-morphological characteristics, this study aims to facilitate the differentiation of closely related species and enhance the taxonomic framework and understanding of their relationships.

The present study focuses on two species formerly placed in *Dorema*, namely, *F. ammoniacum* (D.Don) Spalik, M.Panahi, Piwczyński & Puchałka and *F. aucheri* (Boiss.) Piwczyński, Spalik, M.Panahi & Puchałka providing micromorphological evidence supporting their inclusion within *Ferula*, as previously suggested by molecular data (Panahi *et al.* 2018, Puchałka *et al.* 2023). Through detailed examination of epidermal and stomatal features, this study provides additional micromorphological evidence that supports the phylogenetic reassessment of *Dorema* species within Ferulinae, as clarified by Puchałka *et al.* (2023), thereby contributing to a better understanding of the taxonomy and biodiversity of *Ferula*.

Materials and Methods

- Plant specimens

The studied specimens were obtained from the herbarium collections of HUI and SFAHAN. Taxonomic identification was carried out using the diagnostic keys and morphological descriptions provided by Chamberlain & Rechinger (1987) and Mozaffarian (2007). For each species, 1–3 herbarium specimens were examined and at least three mature leaves per specimen were selected for micromorphological analysis. Species selection was based on the availability of herbarium material and aimed to reflect the morphological diversity of the genus, with emphasis on the flora of central and southern Iran. Detailed information on the studied taxa and voucher specimens is presented in table 1.

Table 1. List of the studied species in Iran and their corresponding herbarium voucher information. The asterisk (∗) indicates the samples that were additionally used for SEM observations [HUI: Herbarium of the University of Isfahan; SFAHAN: Herbarium of the Isfahan Agricultural and Natural Resources Research and Education Center, Isfahan, Iran.]

Taxon	Locality	Alt. (m)	Collection date	Collector	Voucher and herb. No.
Ferula ammoniacum (D.Don) Spalik, M.Panahi, Piwczyński & Puchałka	Isfahan Prov.: Isfahan to Abadeh	2000	03.08.2008	Rahimzadeh & Aryavand	HUI-16744*
	Isfahan Prov.: Anarak	1750–1800	12.04.1993	Feyzi	SFAHAN-9186
	Markazi Prov.: Delijan		06.05.1994	Shams & Feyzi	SFAHAN-9887
F. assa-foetida L.	Isfahan Prov.: Semirom, Vanak	1700	17.05.2001	Parishani	HUI-14017*
	Isfahan Prov.: Fereydunshahr, Mount Hashtad	1700	11.07.2023	Bagheri	HUI-24036
	Isfahan Prov.: Ardestan	-	03.05.2009	Feyzi	SFAHAN-15055
F. aucheri (Boiss.) Piwczyński, Spalik, M.Panahi & Puchałka	Kohgiluyeh and Boyer-Ahmad Prov.: Yasuj, 4 km to Kakan	2350	25.05.2005	Shams	SFAHAN-14794*
	Isfahan Prov.: Tiran, Dalan Kuh	2600	29.05.2021	Shahzeidi	HUI-24040

Table 1 (contd)

F. gummosa Boiss.	Isfahan Prov.: Semirom	2550	24.05.1984	Aryavand & Sahebi	HUI-10083*
	Isfahan Prov.: Khansar	2500	15.06.1992	Feyzi	SFAHAN-8873
	Tehran Prov.: Tehran to Damavand	2550	16.07.2012	Rasti & Moosaie	SFAHAN-7786
F. haussknechtii H.Wolff ex Rech.f.	Isfahan Prov.: Fereydunshahr, Hashtad Mt.	3200	15.06.2023	Bagheri	HUI-24037*
	Isfahan Prov.: Khansar	2500	07.03.2017	Sabouri	HUI-22416
	Kohgiluyeh and Boyer-Ahmad Prov.: Loodab	2400	05.05.2015	Aminimehr	HUI-21882
F. hirtella Boiss.	Isfahan Prov.: Shahreza to Abadeh	1987	20.05.2022	Shahzeidi	HUI-24042
	Isfahan Prov.: Isfahan, Naein to Jandagh	1050	04.05.1993	Feyzi	SFAHAN-9316
	Isfahan Prov.: Meymeh to Muteh	1920	22.06.2002	Feyzi	HUI-13111*
F. kashanica Rech.f.	Isfahan Prov.: Najafabad, Dehagh	2100	16.05.2000	Feyzi & Shams	SFAHAN-12579*
F. microcolea (Boiss.) Boiss.	Isfahan Prov.: Fereydan, Qaleh Bahman	2500	15.05.2000	Feyzi & Shams	SFAHAN-12616
	Isfahan, Fereydan	2400	16.05.2000	Feyzi & Shams	SFAHAN-12448*
	Isfahan Prov.: Daran, Bouin, Darr-e Hooz	2550–2600	18.06.2003	Norouzi & Shams	SFAHAN-13870
F. oopoda (Boiss. & Buhse) Boiss.	Isfahan Prov.: Mimeh, Mouteh, NW of Robat Tork	2050	11.06.2002	Feyzi	SFAHAN-13161
	Isfahan Prov.: Meymeh, Das Pass	1950–2000	21.05.2015	Akhavan & Bagheri	SFAHAN-17016*
	Isfahan Prov.: Ghalesiah to Hosseinabad	1750–1850	30.05.1989	Feyzi & Norouzi	SFAHAN-6060
F. ovina Boiss.	Isfahan Prov.: Semirom, Vanak	2500	04.06.2001	Parishani	HUI-14018*
	Isfahan Prov.: Kashan	1020	21.05.1997	Esmaeili	HUI-2540
	Isfahan Prov.: Natanz	1700	06.05.2009	Afsharzadeh & Abbasi	HUI-17502
F. persica Willd.	Isfahan Prov.: Fereydun	2400	16.05.2000	Feyzi & Shams	SFAHAN-12477*
	Alborz Prov.: Beginning of Karaj-Chalus Road from Karaj	1450	08.05.2022	Shahzeidi	HUI-24041
F. stenocarpa Boiss. & Hausskn.	Fars Prov.: Dehram, Namaki Cave	100	26.04.2023	Shahzeidi	HUI-24038
	Bushehr Prov.: Behbahan to Bandar-e Deylam	900	09.03.2007	Feyzi	SFAHAN-14931*
	Hormozgan Prov.: Bandar Abbas, Geno Mt.	100	10.04.1992	Rasouli	SFAHAN-8225
F. szowitsiana DC.	Isfahan Prov.: Naein, Seifabad	-	21.04.1982	Aryavand	HUI-6036*

Table 1 (contd)

Leutea cupularis (Boiss.) Pimenov	Isfahan Prov.: Semirom, Dolat Gharin Village, Sarzeh Mt.	3172	25.07.2019	Shahzeidi	HUI-24044
	Isfahan Prov.: Semirom, Ab Malakh Village	1950	25.07.2019	Shahzeidi	HUI-24043*
L. petiolaris (DC.) Pimenov	Isfahan Prov.: Khansar, Bouin	2830	15.07.2019	Akhavan & Bagheri	SFAHAN-17143*
	Isfahan Prov.: Khansar, Bouin	2830	15.07.2019	Akhavan & Bagheri	SFAHAN-17142
	Mazandaran Prov.: Seranza	2100	07.09.2021	Afsharzadeh & Shahzeidi	HUI-24045

- Anatomy of leaf epidermis

One to three specimens per species were used. Leaves were softened by immersion in a solution of ethanol and glacial acetic acid (3:1) for 24 h (modified from Metcalfe & Chalk 1950). Subsequently, samples were placed in a 1:1 solution of hydrogen peroxide and acetic acid and incubated on a hot plate at 70–85 °C for 0.5–3 h for bleaching and clearing. After rinsing with distilled water, the abaxial epidermis was carefully separated using a scalpel and fine forceps, mounted on microscope slides, and observed under an Olympus BX-50 light microscope at magnifications of 10×, 20×, and 40×. Images were captured using a digital camera with reference scales of 60, 30, and 200 µm. For each specimen, stomata were counted in three non-overlapping fields, each covering 0.074 mm². Stomatal density (number/mm²) was calculated as the mean number of stomata per field divided by the area.

- Scanning electron microscopy (SEM) analysis

Leaf segments were mounted on aluminum stubs with double-sided conductive tape and sputter-coated with a 10 nm gold layer. Samples were examined using a scanning electron microscope (SEM) at the Faculty of Materials Engineering and Metallurgy, Isfahan University of Technology (Isfahan, Iran). Images were obtained at magnifications corresponding to 200, 100, 50, and 20 µm scales. The specimens used for SEM analysis were marked with an asterisk in table 1.

- Stomatal counting and analysis

Stomatal counts, epidermal cell counts, and stomatal index were obtained from LM images taken at a 30 µm scale. The paradermal area of each image was calculated, and stomatal index was determined using the formula (Salisbury 1928): Stomatal Index = 100 × [number of stomata / (number of stomata + number of epidermal cells)].

Counts were performed using Digimizer software v.5.4.9. Descriptive statistics including the standard error of the mean (SEM) for stomatal length and width were calculated using Microsoft Excel. Scatter plots illustrating the relationships between stomatal length and width, as well as between stomatal density and stomatal index, were produced using R software, including standard error bars.

Results

- Stomatal types and epidermal cell shapes

Based on light microscopy observations (Fig. 1), two main types of stomata were identified on the abaxial surface of the leaves i.e., anisocytic and anomocytic. Anisocytic stomata were predominant across most species, although a few anomocytic stomata were also observed at low frequency in several taxa. Notably, *F. gummosa* showed a predominance of anomocytic stomata. The shape of the epidermal cells varied from polygonal to irregular, or a combination of both. In most *Ferula* species, cells were predominantly irregular, while *Leutea* species showed both polygonal and irregular forms.

- Stomatal dimensions

The mean stomatal length ranged from 21.61 µm in *F. ovina* to 37.31 µm in *F. stenocarpa*, while stomatal width ranged from 14.93 µm in *F. oopoda* to 29.49 µm in *F. ovina*. *F. microcolea* and *F. stenocarpa* displayed relatively longer stomata, whereas shorter and narrower stomata were found in *F. kashanica* and *F. oopoda*. Variation in stomatal dimensions was evident among species, contributing to their taxonomic differentiation (Ostroumova 1990, Zhou & Liu 2018).

- Stomatal density and stomatal index

Stomatal density values ranged from 54.05 in *F. oopoda* to 216.22 in *F. assa-foetida*. The stomatal index showed a comparable trend, ranging from 6.19% in *L. cupularis* to 15.87% in *F. stenocarpa*. In general, species with higher stomatal density also exhibited higher stomatal index, although exceptions were noted in *F. ovina* and *F. gummosa*. Detailed values of stomatal traits are provided in table 2.

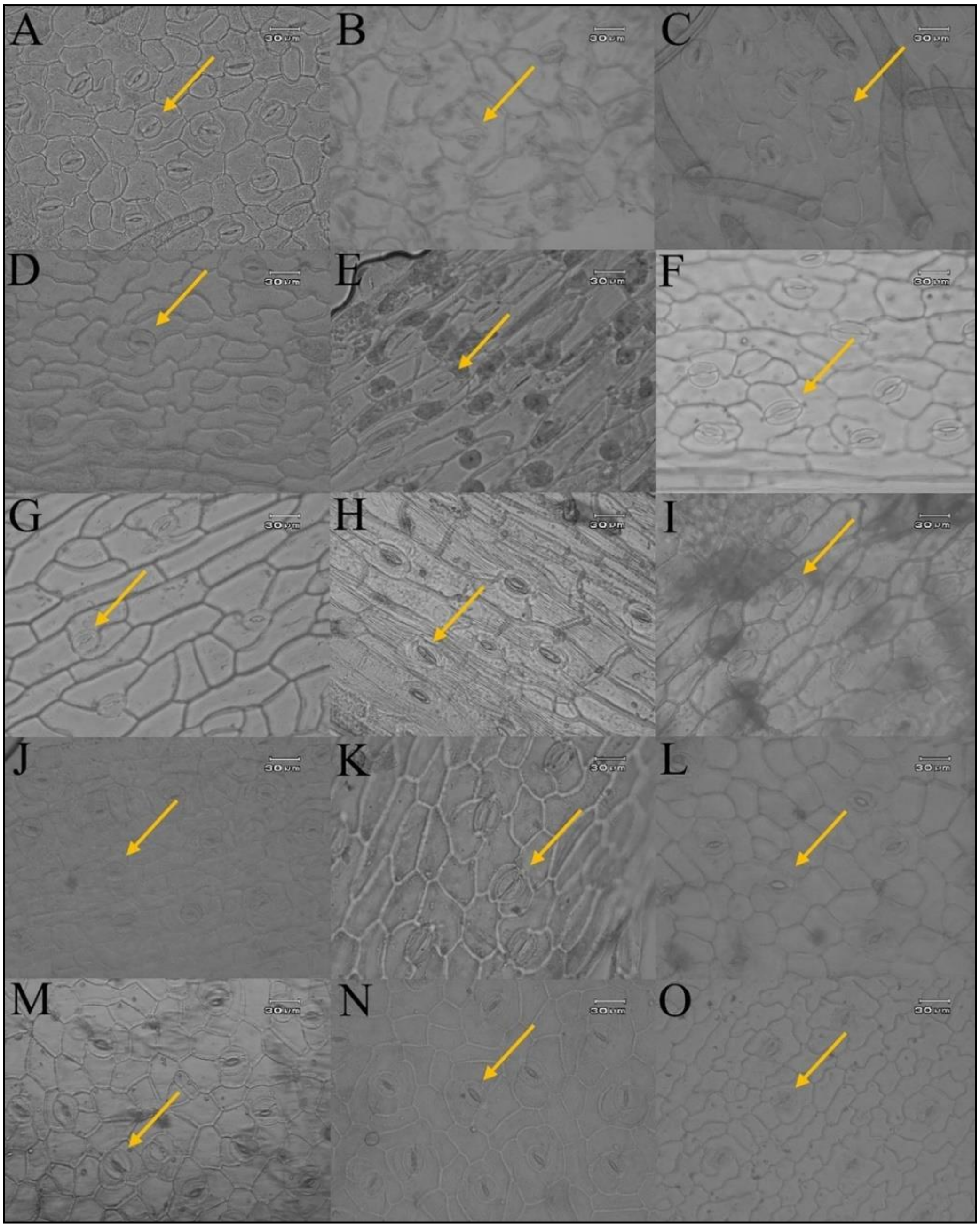

Fig. 1. LM of the shapes of the epidermal cells on the abaxial surface and the arrangement of the cells surrounding the guard cells of different species: A. *Ferula assa-foetida*, B. *F. ovina*, C. *F. szowitsiana*, D. *F. haussknechtii*, E. *F. gummosa*, F. *F. stenocarpa*, G. *F. oopoda*, H. *F. persica*, I. *F. hirtella*, J. *F. kashanica*, K. *F. microcolea*, L. *F. aucheri*, M. *F. ammoniacum*, N. *Leutea petiolaris*, O. *L. cupularis* (Bar = 30 µm).

Table 2. Overview of epidermal cell and stomatal traits in the studied species, including sample sizes (N), mean values, standard deviations (SD), and standard error of the mean (SEM) for stomatal length and width, as well as stomatal density (number/mm²) and stomatal index (%)

Taxon	Sample size	Mean stomatal length (µm)	SD of stomatal length (µm)	SEM of stomatal length (µm)	Mean stomatal width (µm)	SD of stomatal width (µm)	SEM of stomatal width (µm)	Epidermal cell shape	Epidermal projection shape	Stomatal density (per mm²)	Stomatal index (%)
Ferula ammoniacum	9	25.89	6.24	2.08	20.75	6.49	2.16	Polygonal	-	175.68	10.92
F. assa-foetida	9	28.42	2.15	0.72	19.80	3.52	1.17	Polygonal, Irregular	striate	216.22	13.91
F. aucheri	9	29.48	3.27	1.09	26.19	5.77	1.92	Irregular, Polygonal	striate	108.11	8.33
F. gummosa	9	28.52	7.93	2.64	16.60	5.25	1.75	Polygonal, Irregular	Smooth	94.59	13.46
F. haussknechtii	9	25.34	9.39	3.13	18.59	7.57	2.52	Irregular	striate	108.11	9.64
F. hirtella	9	28.63	7.59	2.53	19.85	6.15	2.05	Polygonal, Irregular	striate, Smooth	94.59	14.93
F. kashanica	3	21.85	1.72	0.57	16.99	6.92	2.31	Irregular	wax-covered	202.70	11.03
F. microcolea	9	31.09	4.21	1.40	22.76	1.79	0.60	Polygonal	striate	81.08	10.17
F. oopoda	9	25.14	7.46	2.49	14.93	5.86	1.95	Polygonal	wax-covered	54.05	8.89
F. ovina	9	21.61	8.62	2.87	29.49	14.44	4.81	Irregular	striate	121.62	13.56
F. persica	6	29.07	8.23	2.74	21.92	6.05	2.02	Irregular	Smooth	94.59	15.69
F. stenocarpa	9	37.31	5.36	1.79	22.05	4.62	1.54	Irregular	Smooth	135.14	15.87
F. szowitsiana	3	26.83	3.56	1.19	18.07	7.06	2.35	Polygonal, Irregular	striate	121.62	12.70
Leutea cupularis	6	21.95	3.65	1.22	17.85	4.17	1.39	Polygonal, Irregular	striate	94.59	6.19
L. petiolaris	9	26.16	7.92	2.64	16.32	5.58	1.86	Irregular, Polygonal	striate	162.16	12.73

- Epidermal surface micromorphology (SEM analysis)

SEM analysis (Fig. 2) revealed three main types of surface ornamentation on the epidermal cell walls: striate, smooth, and wax-covered. Striate ornamentation was the most frequent, occurring in the majority of species. Smooth surface projections were found in *F. gummosa*, *F. persica*, and *F. stenocarpa*. The wax-covered type likely corresponds to epicuticular secretions rather than true glands, as interpreted from SEM images and following Barthlott (1981), as seen in *F. kashanica*, *F. oopoda*, and *F. stenocarpa*. In *F. ammoniacum*, *L. petiolaris*, and *L. cupularis* (Fig. 2M-O), the boundaries of epidermal cells were distinctly visible. In most other species, however, these boundaries appeared less prominent or were difficult to distinguish. The SEM observations supported and complemented the findings from light microscopy, increasing the resolution of micromorphological differences among the studied taxa.

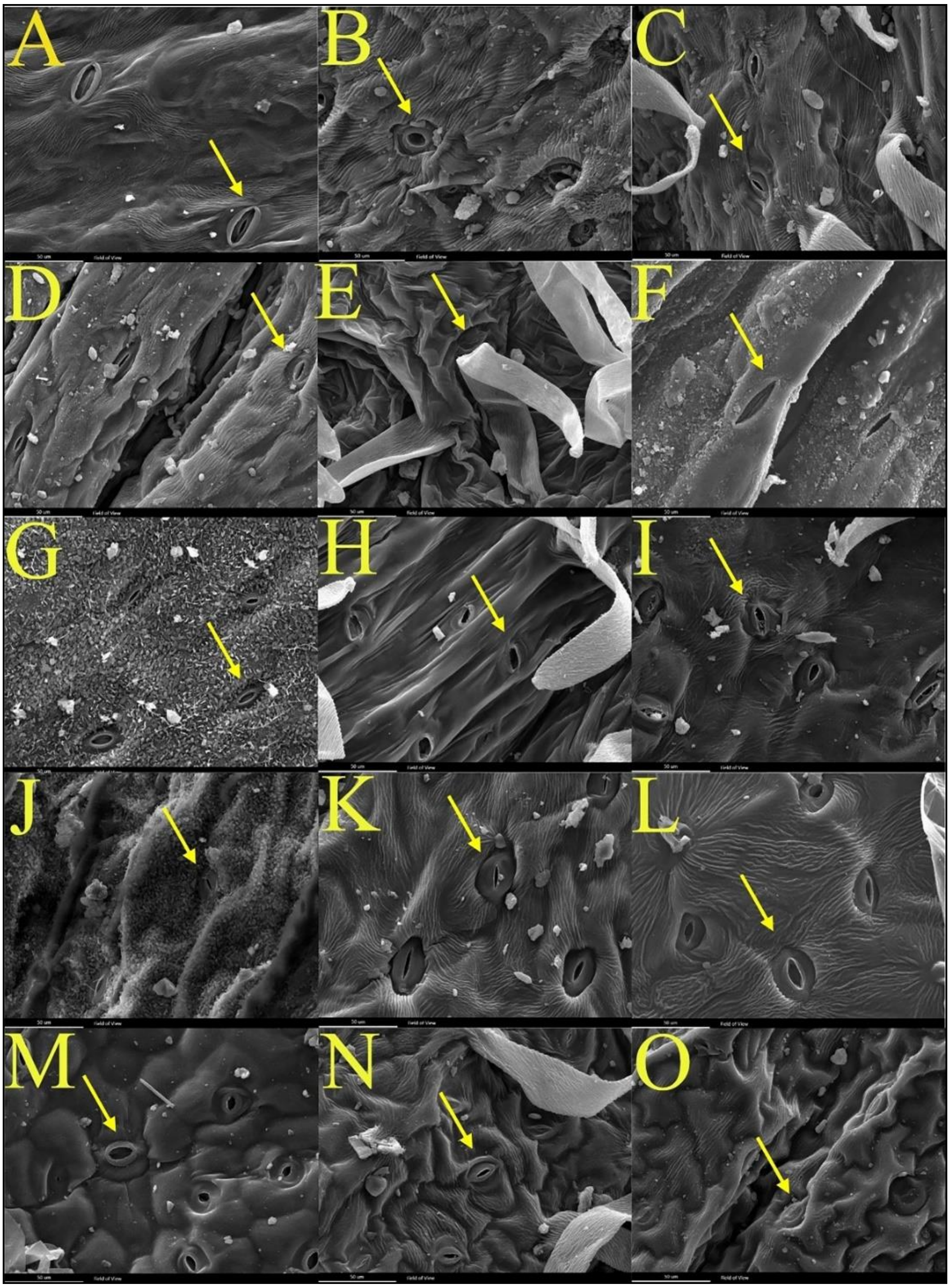

Fig. 2. SEM of the surface projections on the epidermis and detailed measurements of stomatal length and width in different species: A. *Ferula assa-foetida*, B. *F. ovina*, C. *F. szowitsiana*, D. *F. haussknechtii*, E. *F. gummosa*, F. *F. stenocarpa*, G. *F. oopoda*, H. *F. persica*, I. *F. hirtella*, J. *F. kashanica*, K. *F. microcolea*, L. *F. aucheri*, M. *F. ammoniacum*, N. *Leutea petiolaris*, O. *L. cupularis* (Bar = 50 µm).

- Relationships between stomatal characters

Scatter plot analyses were performed to assess the relationships between key stomatal traits. A positive correlation was observed between stomatal density and stomatal index across the studied species, although a few exceptions occurred. Similarly, species with longer stomata often exhibited wider stomatal apertures, with notable variation in *F. ovina* and *F. microcolea*. Figures 3–4 illustrate these patterns, with error bars in figure 3 indicating intra-species variability. These visualizations supported the numerical findings and indicated observable variation in stomatal traits among the examined species.

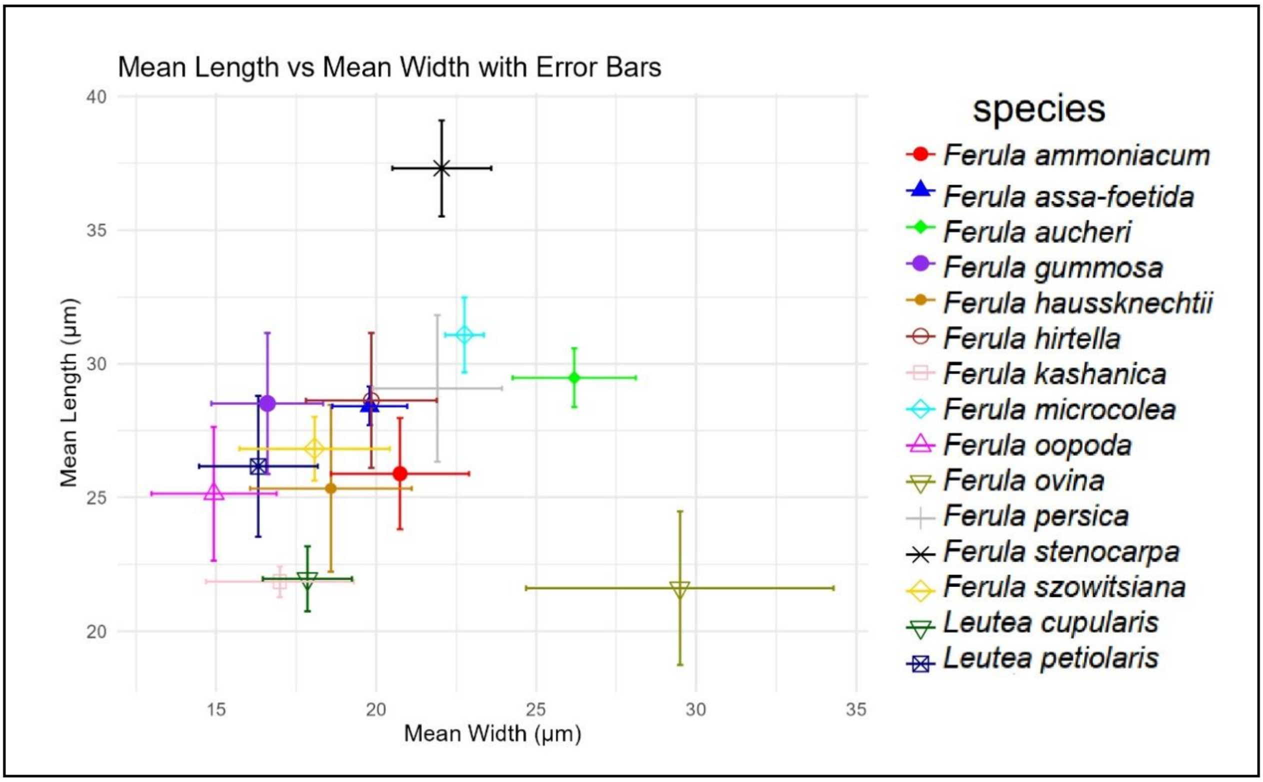

Fig. 3. Scatter plot illustrating the relationship between stomatal length and stomatal width among the studied species. Error bars represent the standard error of the mean.

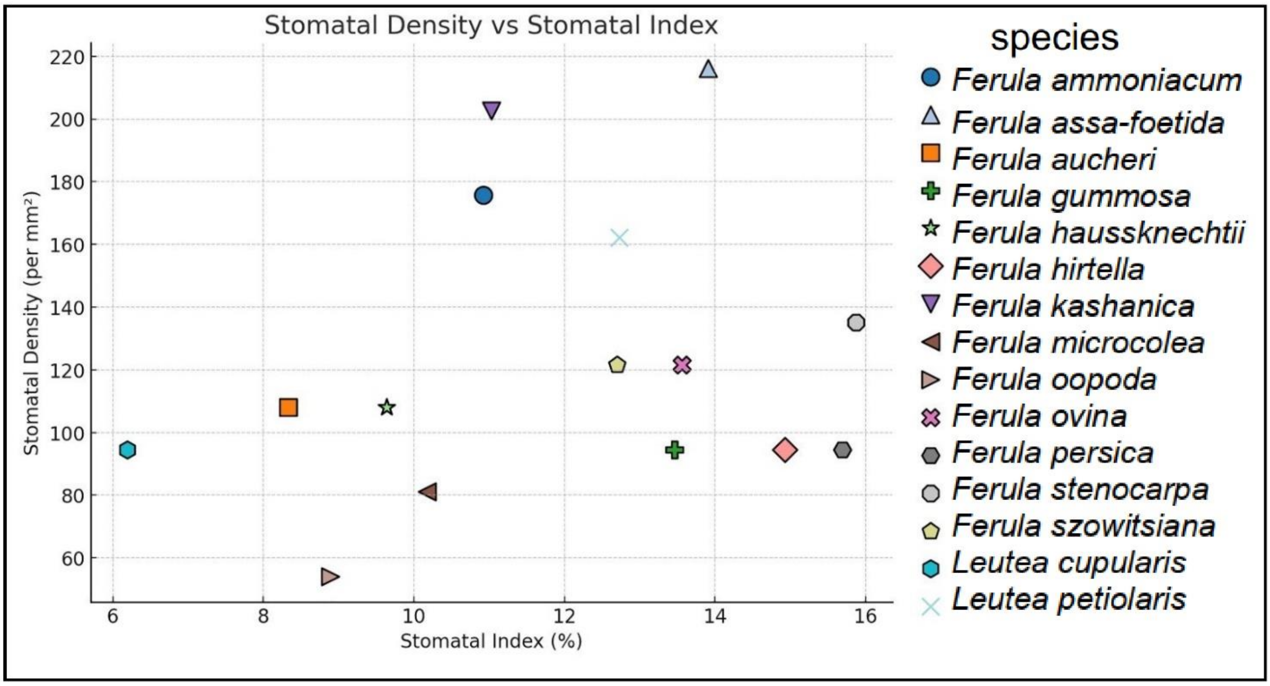

Fig. 4. Scatter plot illustrating the relationship between stomatal density (number of stomata per mm²) and stomatal index (%) in the studied species.

Discussion

Micromorphological features of the leaf epidermis have proven useful in taxonomic studies of *Ferula* and allied genera (Ostroumova 1990, Plunkett *et al.* 2018, Zhou & Liu 2018). In the present study, variations in stomatal length, width, and index, as well as epidermal configurations, were analyzed to evaluate their taxonomic value. The predominance of anisocytic stomata in most species, with the exception of anomocytic types observed in *F. gummosa*, supports the taxonomic relevance of these traits in species delimitation. The observed variation in stomatal dimensions and indices provided supportive evidence for distinguishing species and highlighted the potential usefulness of these traits in taxonomic assessments. Differences in epidermal cell shapes and ornamentation contributed additional resolution, particularly for closely related or morphologically similar taxa. Scatter plots (Figs 3–4) were generated to examine the relationships between stomatal length and width, and between stomatal density and stomatal index. These visualizations, including error bars, reflected measurable variation among the studied species and allowed detection of outlier taxa such as *F. stenocarpa* and *L. cupularis*. The distinct position of *F. stenocarpa*, collected from hot and arid lowlands, and *L. cupularis*, from humid highlands of Semirom, suggests that, environmental conditions can significantly influence stomatal traits. No clear pattern was observed between stomatal density and stomatal index among the studied taxa (Fig. 4), indicating that, these traits are likely influenced by complex interactions of genetic, physiological, and environmental variables. This lack of uniformity supports the notion that micromorphological characters must be evaluated in context and not in isolation. The results of the present study align with earlier findings by Ostroumova *et al.* who emphasized the significance of stomatal types and cell arrangements in resolving taxonomic relationships in Apiaceae. For example, stomatal configurations have been reported as important markers for distinguishing tribes such as Coriandreae and Scandiceae (Ostroumova & Kljuykov 2007), and variation within subtribes like Foeniculinae has also been demonstrated (Ostroumova 1990, Ostroumova & Lavrova 1991). The micro-morphological evidence gathered here reinforces the diagnostic value of stomatal traits between *Ferula* and its close relative *Leutea*, although these traits alone are insufficient to clearly differentiate species within each genus. Comparative evidence from other genera of Apiaceae further supports these findings. In *Ligusticum*, consistent epidermal characters were shown to enhance taxonomic resolution (Zhou & Liu 2018), while in *Pimpinella* and *Sanicula*, micromorphological features such as cuticular ornamentation and anticlinal wall patterns have proven diagnostic (Wang *et al.* 2022, Song *et al.* 2024). The congruence of these results across multiple genera confirms the broader taxonomic value of micromorphological traits in Apiaceae. In this study, the types of ornamentation observed, striate, smooth, and wax-covered, were correlated to some extent with phylogenetic lineages suggested by recent molecular studies (Panahi *et al.* 2015). Species sharing similar epidermal traits often clustered within the same molecular clades (Panahi *et al.* 2018, Zhou & Liu 2018, Puchałka *et al.* 2023), suggesting that, these micromorphological features are not only taxonomically informative but may also reflect ecological adaptation. Support for the inclusion of *Dorema* within *Ferula* is reinforced by the shared micromorphological traits observed among former *Dorema* species and members of *Ferula*. These results are in accordance with molecular data (Panahi *et al.* 2015, 2018, Puchałka *et al.* 2023), confirming that, *Dorema* does not represent a distinct genus. The integration of micromorphological and molecular datasets offers a robust framework for resolving long-standing taxonomic ambiguities within the subtribe Ferulinae. In conclusion, although leaf micromorphological features alone may not distinguish all species within *Ferula*, traits such as stomatal configuration, epidermal ornamentation, and stomatal indices significantly enhance species identification. These findings affirm the value of detailed micromorphological analyses in systematic botany and underscore their role as complementary tools along with molecular approaches in elucidating taxonomic relationships and evolutionary patterns within Apiaceae, as also supported by molecular phylogenetic studies of *Ferula* and related genera (Fereidounfar *et al*. 2016).

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