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Micromorphological Study of Epidermis and Stomata in Some *Stachys* L. Species (Lamiaceae) From Iran

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Abstract

Stachys L. is a taxonomically complex and morphologically diverse genus within Lamiaceae, comprising nearly 300 species worldwide, including 34 species reported from Iran. Despite its ecological significance and traditional medicinal uses, the micromorphological characteristics of its leaf surfaces, particularly the epidermis and stomata, have not been thoroughly investigated. In this study, 15 species of *Stachys* were analyzed using light microscopy (LM) and scanning electron microscopy (SEM) to explore their epidermal and stomatal features. Measurements included stomatal length, width, and stomatal index (SI) alongside qualitative assessments of surface ornamentation and stomatal types. The largest stomata were observed in *S. pilosa*, whereas *S. ixodes* had the highest SI and *S. obtusirena* the lowest. All species exhibited irregular epidermal cell outlines and a combination of anisocytic and diacytic stomatal types. These micromorphological characters, particularly when interpreted in combination with other morphological data, provide informative characters that can aid in the taxonomic identification and classification of *Stachys* species.

Keywords: *Stachys*, Micromorphology, Leaf Epidermis, Stomata, Light Microscopy (LM), Scanning Electron Microscopy (SEM)

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Introduction

The mint family, Lamiaceae, includes more than 230 genera and over 7,000 species, making it one of the largest families of flowering plants with a broad global distribution (Harley et al., 2004; Zhao et al., 2021). This family is well known for its characteristic floral structure, square stems, and aromatic members, and the Mediterranean region is still considered one of its primary centers of diversity (Bhattacharjee, 1980).

Among its members, *Stachys* L. stands out due to its high morphological diversity and taxonomic complexity, with more than 300 species described worldwide (Harley et al., 2004; Salmaki et al., 2012). These species range from annual and perennial herbs to subshrubs, exhibiting considerable variation in vegetative and reproductive features (Lindqvist & Albert, 2002). In addition to these traits, many species of *Stachys* have been used in traditional medicine, and various studies have confirmed their antioxidant, antibacterial, and anti-inflammatory properties (Tundis et al., 2014; Giuliani et al., 2008).

In Iran, *Stachys* has been studied in several taxonomic works. Rechinger (1982) recorded 47 species, including 25 endemics, while Jamzad (2012) revised this number to 38. These numbers suggest that Iran plays an important role in the distribution and diversity of the genus in the region. However, separating species based on morphology remains problematic, as many characters' overlap or vary within species. To improve species identification, researchers have turned to micromorphological features, especially those visible under scanning electron microscopy (SEM), such as trichome types (Salmaki et al., 2009), pollen morphology (Salmaki et al., 2008a) and nutlet surfaces (Salmaki et al., 2008b).

Some recent studies from Turkey have also provided valuable data, Dogu (2021) examined the anatomy and pollen of *S. longiflora*, and Gerçek et al. (2022) studied trichomes and secondary compounds in *S. rizeensis* R.Bhattacharjee. Despite this progress, the structure of leaf epidermis and stomata in *Stachys*, especially in Iranian species, has not been studied in detail. These characteristics, such as stomatal size, type, and density, are often stable and less

affected by environmental conditions, which makes them useful for taxonomy.

This study examines these features in 15 Iranian *Stachys* species using both light microscopy (LM) and scanning electron microscopy (SEM). The aim is to provide clear and valuable information to help distinguish species more easily and better understand their variation.

Materials and Methods

Plant material and sample selection

The taxonomy of the selected taxa was reviewed based on established literature (Rechinger, 1982; Jamzad, 2012). The plant specimens utilized in this study were obtained from the herbarium of the Isfahan Agricultural and Natural Resources Research Center (SFAHAN). For each *Stachys* species examined, a minimum of three herbarium specimens from distinct populations was included in the analysis. Detailed information regarding the collected species is presented in Table 1.

Light microscopy (LM)

For light microscopy analysis, a minimum of three samples from each population was examined. Leaves were selected from equivalent positions along the stem to maintain consistency among specimens. The samples were initially immersed in a 3:1 solution of acetic acid and ethanol for 24 to 48 hours to soften the leaf tissues. They were then transferred into a 1:1 mixture of hydrogen peroxide and acetic acid and subjected to heating at 80–100°C for 10 minutes to eliminate pigmentation and enhance the clarity of epidermal structures. Following this treatment, the samples were thoroughly rinsed with distilled water and prepared for epidermal peeling. The method used for preparing and clearing the leaf epidermis followed standard protocols described by Stace (1984) with minor modifications. Given the high density of trichomes, which could obstruct the visibility of stomata, the trichomes were carefully removed using styrofoam. A thin section of the abaxial leaf epidermis was then dissected and mounted on glass slides. The prepared samples were observed and documented

using a light microscope (LM) under 40× magnification.

Table 1. Table 1. Information on the studied *Stachys* specimens, including species names, herbarium numbers, and collection details. Herbarium numbers marked with an asterisk (*) were used for SEM analysis.

Species Name	Herb. NO.	Location, Collector(s), Date
<i>S. acerosa</i> Boiss.	17719	Isfahan, Khansar, Golestankoo; Akhavan & Bagheri, 31 May 2018
	5158	Isfahan, Tiran; Norouzi, 4 May 1987
	14685*	Isfahan, Najafabad, Tiran; Shams, Janighorban, 31 May 2004
<i>S. aucheri</i> Benth.	17720*	Isfahan, Khansar, Golestankoo; Akhavan & Bagheri, 25 June 2018
	10337	Isfahan, Fereydunshahr; Feizi & Asfa, 25 June 1994
	14409	Shahrekord, Saman; Shams & Janighorban, 25 July 2004
<i>S. ballotiformis</i> Vatke	16606*	Isfahan, Fereydunshahr, Poshtkoo; Akhavan & Bagheri, 8 June 2018
<i>S. benthamiana</i> Boiss.	12680	Isfahan, Fereydunshahr; Feizi & Shams, 5 June 2000
	12555*	Isfahan, Golpayegan, Hendeh; Feizi & Shams, 1 May 2000
	2998	Isfahan, Semirom, Padena; Norouzi, 1 June 1983
<i>S. byzantina</i> K.Koch	13718*	Isfahan, Najafabad; Aslani, 24 June 2007
	11243	Mazandaran, Kelardasht; Norouzi, 25 July 1996
<i>S. inflata</i> Benth.	11593	Isfahan, Semirom; Shams, Janighorban & Feizi, 27 May 1998
	4985	Shahrekord, Lordegan; Norouzi & Ebrahimi, 5 June 1986
	17721*	Isfahan, Khansar, Golestankoo; Akhavan & Bagheri, 31 May 2018
<i>S. ixodes</i> Boiss. & Hausskn.	10732	Isfahan, Natanz, Wargoran.; Faizi & Shams 30 May 1995
	14839*	Fars, Eqlid; Norouzi, 13 May 2000
<i>S. kurdica</i> Boiss. & Hohen.	3809	Isfahan, Semirom; Norouzi, 1 May 1985
	3638	Isfahan, Semirom; Norouzi, 13 May 1985
	4851*	Isfahan, Fereydunshahr; Janighorban, 26 May 1986
<i>S. lavandulifolia</i> Vahl.	13322	Isfahan, Fereydan, Bagh Nazar; Norouzi & Yazdani, 27 May 2002
	1003*	Isfahan, Chadegan; Norouzi, Etemadi & Baba Rabie, 22 June 1981
	2431	Isfahan, Shahrekord, Gardaneh Rokh; Norouzi, 16 May 1983
<i>S. obtusicrena</i> Boiss.	15465*	Isfahan, Semirom; Feizi, 6 June 2001
<i>S. pilifera</i> Benth.	14147	Chaharmahal & Bakhtiari, Borujen, Sabzkooh; Norouzi, 1 June 2003
	14662*	Isfahan, Khansar, Fereydan; Shams, Janighorban, 17 June 2004
	2908	Isfahan, Semirom; Norouzi, 10 June 1983
<i>S. pilosa</i> Nutt.	14997*	Isfahan, Fereydunshahr; Nekukho, 11 July 2007
<i>S. setifera</i> C.A.Mey.	10386*	Isfahan, Fereydan; Feizi & Asafa 26 June 1994
	12853	Isfahan, Zarrin Shahr, Bagh Bahadoran; Norouzi, 20 March 2007
	12040	Isfahan, Morche Khort, Meymeh; Norouzi & Shams, 6 September 1998
<i>S. spectabilis</i> Choisy ex DC.	5691*	Chaharmahal & Bakhtiari, Kouhrang, Cheshmeh Dimeh; Norouzi & Akhbari, 13 June 1988
<i>S. tomentosa</i> Benth.	14268	Isfahan, Semirom, Hana; Shams, 1 June 2004
	12774*	Isfahan, Fereydan; Feizi, Shams, 7 June 2000
	12604	Isfahan, Fereydan, Ghal'eh Bahman; Feizi & Shams, 15 May 2000

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was conducted to obtain high-resolution images of leaf surface structures at the Faculty of Materials Engineering, Isfahan University of Technology. Small leaf sections were carefully mounted onto aluminum stubs using double-sided adhesive tape.

To enhance conductivity, a 10-nanometer layer of gold was applied to the samples using a sputter coater. This thin metallic coating did not alter the surface morphology of the samples. Imaging was performed at magnifications of 20×, 50×, 100×, and 200× to visualize fine epidermal features in detail.

Stomatal counting, index calculation, and data analysis

To assess stomatal density and the number of epidermal cells, images captured at 40× magnification under light microscopy were analyzed. The stomatal index (SI) was calculated using the following formula (Zhu et al., 2021):

Stomatal Index = Number of stomata / (Number of epidermal cells + Number of stomata) × 100

Quantitative measurements of epidermal traits were carried out using Digimizer software. Mean values and standard deviations for stomatal length and width were computed, and the data were organized into tables using Microsoft Excel. For each species, the arrangement of epidermal cells and the orientation of guard cells were examined and recorded. To evaluate interspecific differences in stomatal size, a one-way analysis of variance (ANOVA) was applied to stomatal length and width. Tukey's HSD test was used to identify statistically significant differences among species.

Results

In the present study, micromorphological characters of 15 *Stachys* species were analyzed through both quantitative (stomatal length, width, and density) and qualitative (epidermal surface type and stomatal arrangement) assessments. Considerable variation was observed across species in stomatal morphology and epidermal characteristics. Differences in epidermal surface features were noted (Table 2); for instance, glandular epidermal structures were identified in species such as *S. acerosa* and *S. setifera*, whereas others displayed either smooth or ribbed epidermal surfaces. Despite these differences, irregularly shaped epidermal cells were consistently observed in all species. Light microscopy (LM) images (Fig. 1) confirmed these variations, while scanning electron microscopy (SEM) images (Fig. 2) provided detailed visualizations of epidermal ornamentation and stomatal structures. Interspecific differences in stomatal dimensions, stomatal index, and density were also significant (Table 3). The largest stomatal length and width were recorded in *S.*

pilosa, while *S. obtusirena* exhibited the smallest stomatal dimensions. The arrangement of epidermal cells surrounding guard cells was identified as anisocytic and diacytic in all species examined. The highest stomatal index was observed in *S. ixodes*, whereas the lowest value was recorded in *S. obtusirena*. These findings emphasize substantial interspecific variability in stomatal and epidermal micromorphology, highlighting their potential taxonomic relevance in distinguishing *Stachys* species. Statistical analysis supported these observations. One-way ANOVA revealed highly significant differences among species in both stomatal length and width ($p < 0.001$). According to Tukey's HSD test, *S. pilosa*, *S. ixodes*, and *S. tomentosa* exhibited significantly larger stomata compared to species such as *S. acerosa* and *S. inflata*. These results confirm that stomatal dimensions serve as reliable characters for distinguishing species within *Stachys*. The patterns of variation are also illustrated in Fig. 3.

Table 2. Epidermal surface characteristics observed in the studied *Stachys* species.

Species Name	Glandular Epidermis	Smooth Epidermis	Ribbed Epidermis
<i>Stachys acerosa</i>	✓		
<i>Stachys aucheri</i>		✓	✓
<i>Stachys ballotiformis</i>		✓	
<i>Stachys benthamiana</i>			✓
<i>Stachys byzantina</i>		✓	
<i>Stachys inflata</i>		✓	
<i>Stachys ixodes</i>	✓		
<i>Stachys kurdica</i>		✓	✓
<i>Stachys lavandulifolia</i>		✓	
<i>Stachys obtusirena</i>			✓
<i>Stachys pilifera</i>		✓	
<i>Stachys pilosa</i>			✓
<i>Stachys setifera</i>	✓		
<i>Stachys spectabilis</i>		✓	
<i>Stachys tomentosa</i>		✓	

Microscopic images provided a general overview of epidermal and stomatal characteristics in the examined *Stachys* species (Fig. 1). Surface ornamentation, including glandular, ribbed, and smooth epidermal structures, was illustrated

through scanning electron microscopy (SEM) (Fig. 2). Light microscopy (LM) images revealed stomatal types and the arrangement of epidermal cells. Stomatal dimensions were measured based on SEM observations, while the structural features of guard cells were identified using LM.

Table 3. Quantitative stomatal characters were measured in the studied *Stachys* species, including sample size (N), stomatal dimensions (length and width, presented as mean $\pm$ standard deviation and minimum-maximum range), epidermal cell count, and stomatal index (%).

Species	N	Length (μm)	Width (μm)	Epidermal Cells Count	Stomatal Index (%)
<i>S. acerosa</i>	7	28.8 $\pm$ 1.95 (25.37–30.82)	22.8 $\pm$ 1.3 (20.81–23.67)	65	10.9
<i>S. aucheri</i>	5	101.8 $\pm$ 7.54 (92.32–114.31)	75.2 $\pm$ 2.03 (73.37–78.45)	64	7.2
<i>S. ballotiformis</i>	6	87.5 $\pm$ 5.32 (77.41–101.19)	68.5 $\pm$ 2.34 (52.46–77.32)	63	17.8
<i>S. benthamiana</i>	7	44.7 $\pm$ 1.29 (42.69–46.35)	31.2 $\pm$ 1.57 (29.75–34.02)	96	11.1
<i>S. byzantina</i>	7	107.7 $\pm$ 9.52 (92.63–117.13)	68.3 $\pm$ 9.5 (62.95–79.22)	75	17.5
<i>S. inflata</i>	6	19.6 $\pm$ 2.13 (15.64–22.12)	14.8 $\pm$ 2.1 (13.68–18.0)	87	15.5
<i>S. ixodes</i>	6	124.5 $\pm$ 12.15 (119.82–154.1)	106.8 $\pm$ 10.18 (92.87–119.37)	47	24.1
<i>S. kurdica</i>	4	113.6 $\pm$ 5.52 (104.49–119.95)	70.8 $\pm$ 5.1 (63.59–77.64)	22	15.3
<i>S. lavandulifolia</i>	7	28.6 $\pm$ 1.04 (27.67–30.45)	22.3 $\pm$ 1.94 (19.54–25.3)	74	8.6
<i>S. obtusirena</i>	4	95.8 $\pm$ 15.45 (83.11–121.28)	95.9 $\pm$ 15.41 (66.85–99.63)	120	6.9
<i>S. pilifera</i>	6	104.2 $\pm$ 6.2 (98.79–117.67)	72.6 $\pm$ 7.1 (64.33–85.4)	85	14.1
<i>S. pilosa</i>	7	157.4 $\pm$ 10.36 (140.5–173.72)	118.5 $\pm$ 7.34 (110.07–133.16)	90	10.0
<i>S. setifera</i>	6	124.7 $\pm$ 3.5 (122.15–132.04)	81.3 $\pm$ 9.9 (68.73–99.76)	120	12.4
<i>S. spectabilis</i>	6	88.6 $\pm$ 6.14 (78.41–99.25)	66.8 $\pm$ 7.53 (54.73–74.25)	98	13.2
<i>S. tomentosa</i>	6	114.9 $\pm$ 3.15 (104.99–120.41)	74.9 $\pm$ 9.7 (70.68–79.03)	58	14.7

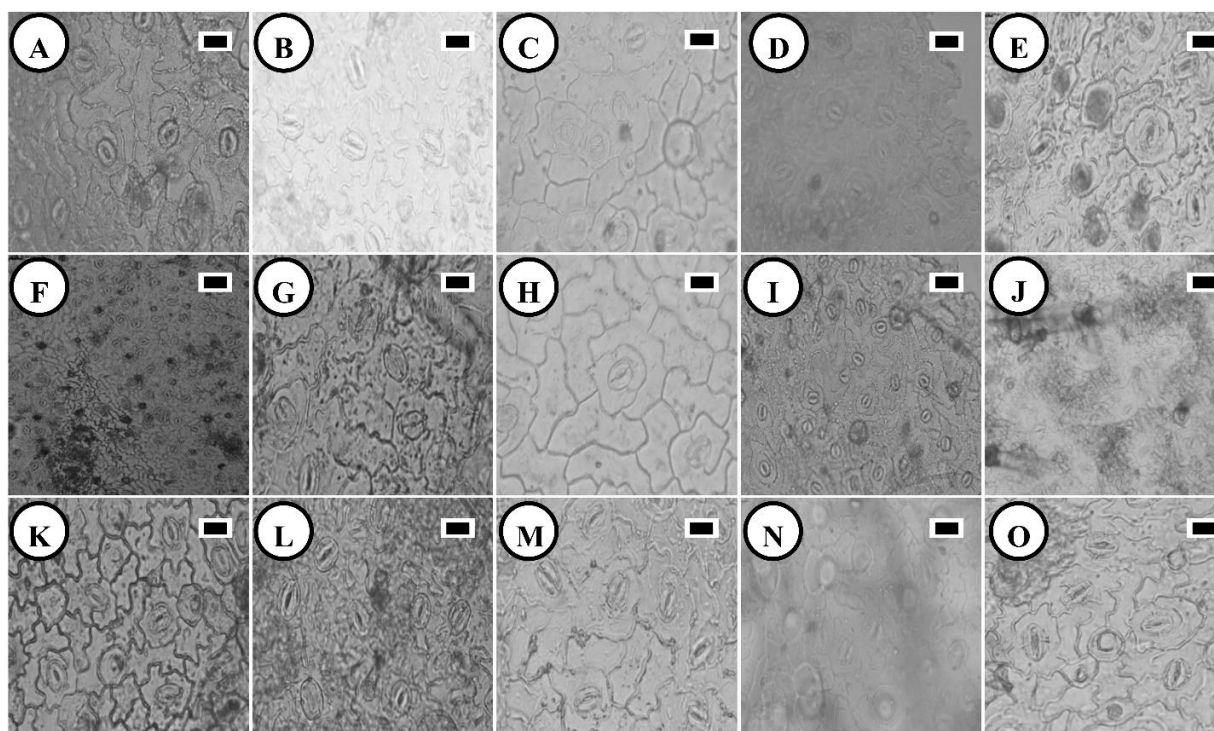


Fig. 1. Light microscopy (LM) images showing epidermal and stomatal structures in selected *Stachys* species. (A) *Stachys acerosa*, (B) *S. aucheri*, (C) *S. ballotiformis*, (D) *S. benthamiana*, (E) *S. byzantina*, (F) *S. inflata*, (G) *S. ixodes*, (H) *S. kurdica*, (I) *S. lavandulifolia*, (J) *S. obtusirena*, (K) *S. pilifera*, (L) *S. pilosa*, (M) *S. setifera*, (N) *S. spectabilis*, (O) *S. tomentosa*. The scale bar corresponds to 30 μm for all species, except in (C), (G), (I), (J), and (M), where it represents 60 μm .

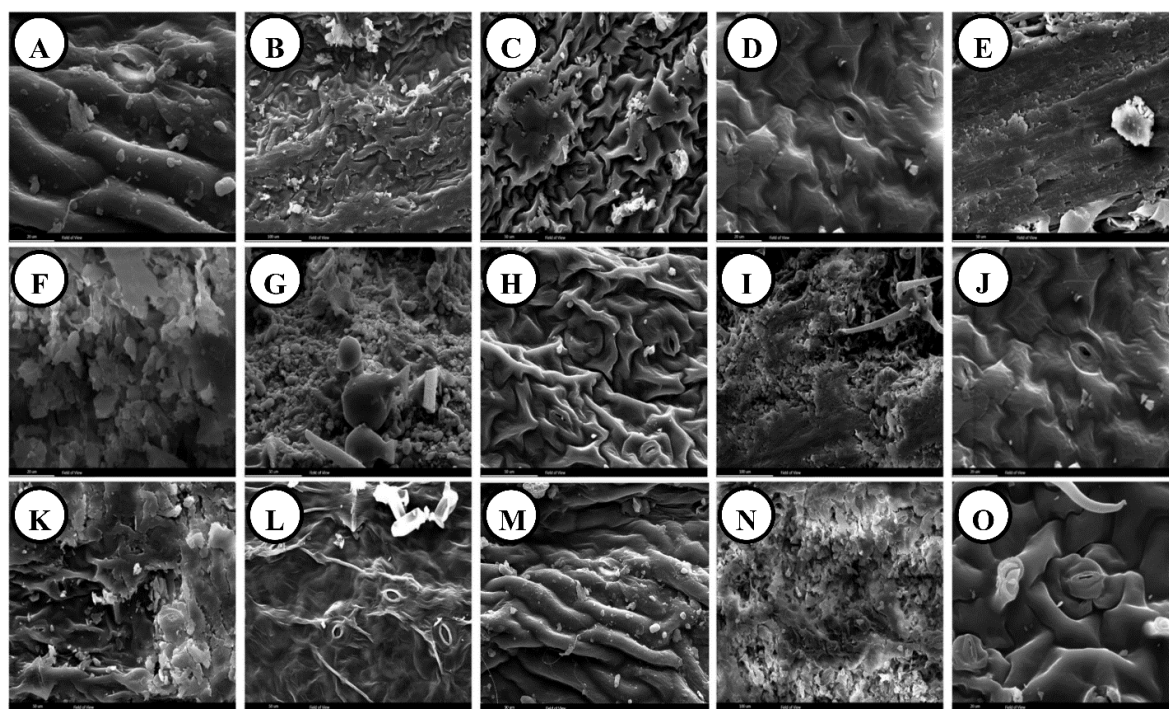


Fig. 2. Scanning electron microscopy (SEM) images of the leaf epidermis in selected *Stachys* species.: (A) *Stachys acerosa*, (B) *S. aucheri*, (C) *S. ballotiformis*, (D) *S. benthamiana*, (E) *S. byzantina*, (F) *S. inflata*, (G) *S. ixodes*, (H) *S. kurdica*, (I) *S. lavandulifolia*, (J) *S. obtusicrena*, (K) *S. pilifera*, (L) *S. pilosa*, (M) *S. setifera*, (N) *S. spectabilis*, (O) *S. tomentosa*. The scale bar represents 50 μm for most species, except for (A), (D), and (F), where it is 20 μm , and (B), (I), and (N), where it is 100 μm .

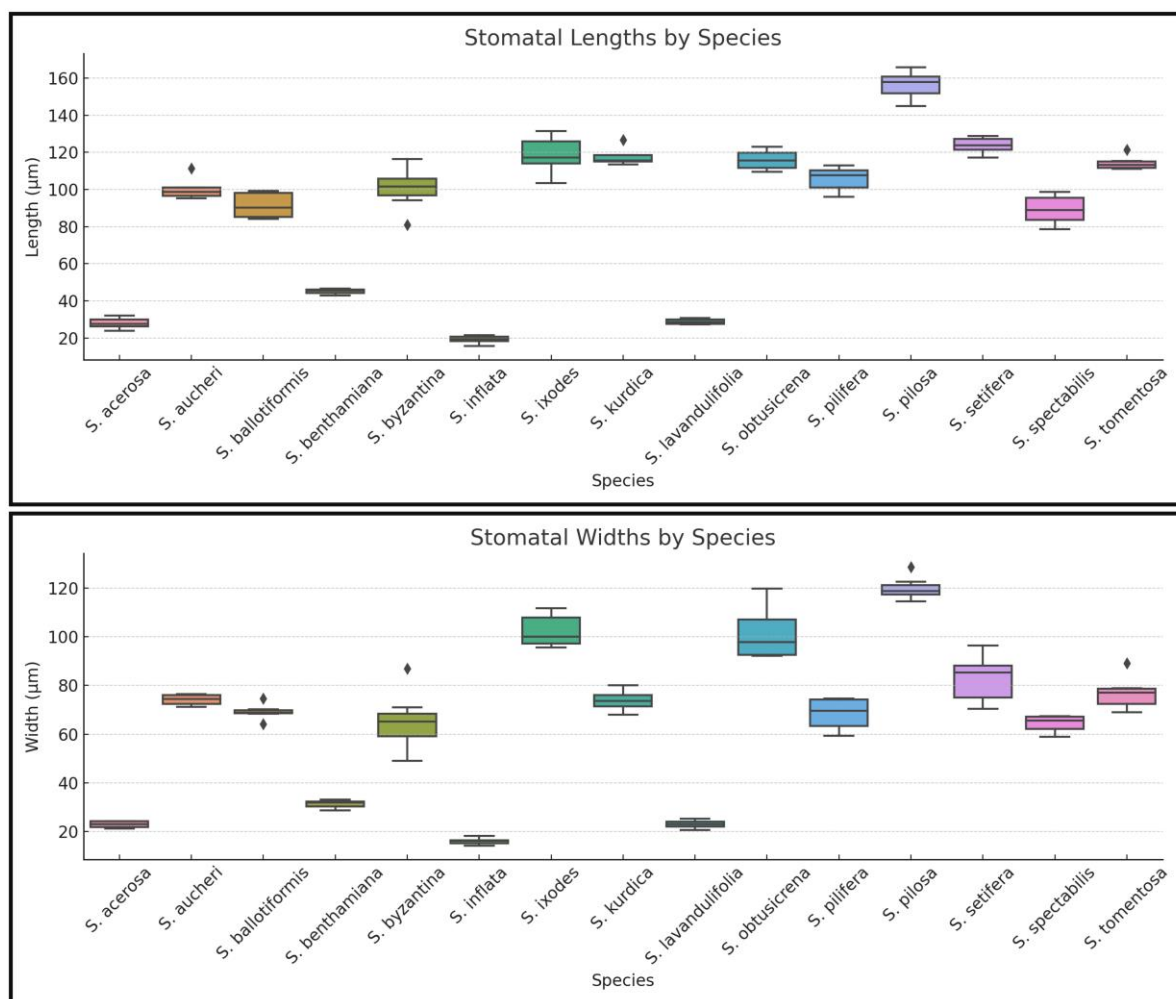


Fig. 3. Boxplots illustrating variation in stomatal length (top) and width (bottom) among the studied *Stachys* species. Each box represents the interquartile range (IQR), with the horizontal line indicating the median value. Whiskers show the range excluding outliers, and dots represent individual outliers.

Discussion

The genus *Stachys* presents a notable taxonomic challenge due to its significant morphological diversity and the difficulty in species delimitation and subgeneric classification. Within the Lamiaceae, anatomical traits have traditionally served as consistent indicators for classification, as they are generally less influenced by environmental variability. Among these characters, foliar micromorphological features, particularly those involving the epidermis and stomata, have received increasing attention for their diagnostic and evolutionary relevance (Gul et al., 2019; Zaman et al., 2022). Despite the recognized medicinal and ecological value of *Stachys*, detailed micromorphological studies, especially on leaf epidermal structures, remain limited in the flora of Iran. This study addresses this knowledge gap by investigating epidermal and stomatal features in 15 Iranian *Stachys* species using light microscopy (LM) and scanning electron microscopy (SEM), with a focus on their taxonomic utility. These micromorphological differences were also statistically supported, confirming their relevance for species-level delimitation within the genus. The results revealed considerable interspecific variation in stomatal characters. *S. pilosa* had the largest stomatal dimensions, while *S. inflata* and *S. acerosa* displayed the smallest. The stomatal index (SI), considered a relatively stable trait less influenced by environmental conditions, ranged from 6.9% in *S. obtusicrena* to 24.1% in *S. ixodes*. These findings are notable when compared to the other genera of the Lamiaceae, such as *Mentha longifolia* (L.) L. (7.3%) and *Micromeria* Benth. (0.4%), highlighting potential adaptive differences (Jabeen et al., 2025). All studied species exhibited irregularly shaped epidermal cells, a feature also reported in other genera of the Lamiaceae, such as *Ocimum* L., *Lavandula* L., *Salvia* L., and *Thymus* L., where it is considered a consistent taxonomic trait (Jabeen et al., 2025; Molaei et al., 2024). Regarding stomatal types, both anisocytic and diacytic stomata were present across all species, consistent with patterns reported in other genera of the Lamiaceae (Gul et al., 2019). Surface ornamentation varied among species, including smooth, ribbed, and glandular types. These characters did not exhibit a clear phylogenetic

distribution but may reflect ecological adaptations. Although trichomes were not the primary focus of the current study, their taxonomic significance is well documented in genera such as *Dracocephalum* L. and *Origanum* L. Previous research has demonstrated that trichome type and density can aid in species discrimination. These findings also align with earlier anatomical studies focusing on nutlets, stems, and petioles (Salmaki et al., 2009; Dogu, 2021), offering a more integrated morphological perspective. While pollen morphology has shown limited taxonomic utility in *Stachys* (Salmaki et al., 2008a), the stomatal and epidermal traits highlighted in this study appear to be more effective in supporting species-level identification. The application of SEM proved especially useful in detecting micromorphological details, particularly in species with dense trichomes that hinder visibility under light microscopy. This methodological advantage has also been recognized in other investigations on Lamiaceae (Semerdjieva et al., 2023). In conclusion, this study highlights the importance of micromorphological traits, particularly those related to stomata and epidermal cells, as valuable diagnostic features in *Stachys*. The integration of these characters with molecular, anatomical, and ecological data is recommended to improve species delimitation and classification within this taxonomically complex genus.

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