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LEAF EPIDERMAL MICROMORPHOLOGY IN TANACETUM (ASTERACEAE) FROM IRANIAN SPECIES: TAXONOMIC IMPLICATIONS

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Abstract
Leaf epidermal micromorphology was investigated in nine *Tanacetum* species from Iran to assess their taxonomic relevance. Both light microscopy (LM) and scanning electron microscopy (SEM) were used to examine qualitative and quantitative characters, including stomatal type, epidermal cell shape, trichome structure, and stomatal measurements. Three stomatal types, namely anomocytic, paracytic, and anisocytic, were observed among the studied taxa, with paracytic and anisocytic stomata restricted to a subset of species. Noticeable variation was also found in stomatal size and stomatal index, as well as in epidermal cell morphology, which ranged from irregular forms in most species to polygonal cells in *T. balsamita*. SEM observations provided clearer details of epidermal surface features and revealed the presence of both glandular and non-glandular trichomes. The combination of these characters allowed differentiation among closely related species and highlighted patterns that are not always evident at the macromorphological level. The results indicate that micromorphological traits of the leaf epidermis can provide useful complementary evidence for species delimitation in *Tanacetum*.

Keywords: Compositae; leaf epidermis; micromorphology; stomata; trichome; taxonomy

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ریزریخت‌شناسی اپیدرم برگ در *Tanacetum* (Asteraceae) و کاربرد آن در رده‌بندی گونه‌های ایرانی

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چکیده: ریزریخت‌شناسی اپیدرم برگ در ۹ گونه از جنس *Tanacetum* از ایران به منظور بررسی ارزش رده‌بندی این صفات مطالعه شد. برای این منظور، از میکروسکوپ نوری (LM) و میکروسکوپ الکترونی روبشی (SEM) جهت ارزیابی ویژگی‌های کیفی و کمی شامل نوع روزنه، شکل سلول‌های اپیدرمی، ساختار کرک‌ها و اندازه‌گیری صفات روزنه‌ای استفاده گردید. نتایج نشان داد که در گونه‌های بررسی‌شده سه نوع روزنه آنوموسیتیک، پاراسیتیک و آنیزوسیتیک وجود دارد و روزنه‌های پاراسیتیک و آنیزوسیتیک تنها در برخی گونه‌ها مشاهده شدند. همچنین تفاوت‌های قابل توجهی در طول و عرض روزنه، شاخص روزنه و الگوی سلول‌های اپیدرمی مشاهده شد. در اغلب گونه‌ها سلول‌های اپیدرمی نامنظم بودند، در حالی‌که در *T. balsamita* سلول‌های چند ضلعی با سطح صاف دیده شد. بررسی‌های SEM جزئیات دقیق‌تری از سطح اپیدرم آشکار ساخت و حضور کرک‌های غده‌ای و غیرغده‌ای را به‌خوبی نشان داد. مجموعه صفات مورد مطالعه امکان تفکیک گونه‌های نزدیک به یکدیگر را فراهم کرد و برخی تفاوت‌ها را آشکار نمود که در صفات ریخت شناسی به‌سادگی قابل تشخیص نیستند. بر اساس نتایج به‌دست‌آمده، ویژگی‌های ریزریخت‌شناختی اپیدرم برگ می‌توانند به‌عنوان شواهدی مکمل و سودمند در تعیین حدود گونه‌ها و مطالعات رده‌بندی جنس *Tanacetum* مورد استفاده قرار گیرند.

INTRODUCTION

The family Asteraceae, with more than 1,700 genera and approximately 25,000 species, is recognized as one of the largest and most widely distributed families of flowering plants (Fitriyati & al. 2024). This broad distribution has been associated with high morphological diversity and considerable ecological adaptability (Zhang & Elomaa 2024; Costas & al. 2024). Within the tribe Anthemideae, the genus *Tanacetum* L. represents an important taxonomic group, comprising about 200 species worldwide. Members of this genus are mostly annual or perennial herbaceous plants and are widely distributed across the Northern Hemisphere, with major centers of diversity in the Mediterranean region, Central Asia, and Southwest Asia (Mandel & al. 2019; Criado-Ruiz & al. 2025). In addition to its taxonomic importance, *Tanacetum* has notable economic and medicinal value. Some species have been used as natural insecticides and repellents, particularly *T. cinerariifolium* (Trevir.) Sch.Bip., which is a well-known source of commercial botanical insecticides (Pavela 2016). Other species, such as *T. vulgare* L., have been traditionally applied in companion planting and as natural preservatives. From a phytochemical perspective, the genus is rich in bioactive compounds. Sesquiterpene lactones, especially parthenolide in *T. parthenium* (L.) Sch.Bip., are associated with anti-migraine and anti-inflammatory effects. At the same time, essential oils containing compounds such as camphor, borneol, and flavonoids in species like *T. balsamita* L. and *T. vulgare* have shown antimicrobial and antioxidant activities (Nadaf & al. 2025; Zandi & al. 2023). Taxonomic studies in Anthemideae have traditionally relied on macromorphological characters, including inflorescence structure, capitula features, and leaf and fruit morphology (Azani & al. 2009). However, the reliability of these traits is often limited due to convergent evolution and environmentally induced variation, which may obscure species boundaries and phylogenetic relationships. For this reason, micromorphological characters have increasingly been considered as complementary and, in some cases, more stable sources of taxonomic information. In particular, the examination of surface structures using scanning electron microscopy (SEM) has provided detailed and less variable characters. Features such as trichomes and leaf epidermal structures have been shown to possess diagnostic value within Anthemideae (Chehregani & Mahanfar 2007; Karbalaei & al. 2021). In recent years, micromorphological studies on several genera of Asteraceae, including *Artemisia* L., *Achillea* L., and *Tanacetum* L., have contributed to the clarification of intrageneric relationships. At the same time, biosystematic investigations in Iran and other regions have emphasized the importance of integrating morphological and micromorphological data. For example, studies on *Helianthus tuberosus* L. (A'la & al. 2023) and *Artemisia* (Karbalaei & al. 2021) have demonstrated the taxonomic value of morphological traits such as stem and leaf characteristics and indumentum. In addition, micromorphological analyses in genera such as *Crepis* L. (Hassan & al. 2024), and species like *Aster glehnii* F.Schmidt (Zaman & al. 2024) have confirmed the usefulness of traits such as stomatal features, epidermal cell patterns, and trichome types in species delimitation. Similar results have been reported for *Tanacetum* species in Turkey, where micromorphological traits of leaf surfaces and trichomes were shown to be taxonomically informative

(Dere & Akcin 2017). Moreover, palynological studies on Anthemideae (Olanj & al. 2017) and *Cousinia* Cass. (Sadeghi Rashti & al. 2013) have highlighted the role of pollen micromorphology in clarifying relationships, a finding supported by other studies (Atazadeh & Ghahremaninejad 2025; Usma & al. 2022; Mahmoudi & al. 2018; Shabestari & al. 2013). In Iran, 31 species of *Tanacetum* have been reported (Mozaffarian 2008). In *Flora Iranica*, the genus was divided into 18 sections mainly based on morphological characters (Podlech 1986). However, sectional assignment was not clearly indicated for all Iranian taxa in the available treatments. Based on this background, the present study was conducted to provide a comparative micromorphological analysis of selected *Tanacetum* species from Iran. The examined taxa were selected from available herbarium material to represent part of the morphological diversity of the genus and several infrageneric groups recognized in *Flora Iranica*. Key epidermal features were examined using light microscopy (LM) and scanning electron microscopy (SEM) to evaluate their possible taxonomic significance.

MATERIALS AND METHODS

Sampling

Leaf samples were obtained from 26 herbarium specimens representing nine species of *Tanacetum*. For each specimen, three replicates (leaf samples) were prepared and examined in order to reduce observational error and to improve the reliability of the measurements. The specimens were deposited in the Herbarium of the Isfahan Agricultural and Natural Resources Research Center (SFAHAN) and the Herbarium of the University of Isfahan (HUI), Isfahan, Iran (Table 1). Species identification was carried out using morphological characteristics, and taxa were identified according to the Flora of Iran (Mozaffarian 2008).

Table 1. Collecting data on the examined *Tanacetum* species.

Taxa	Locality, collector & Voucher No.
Sect. *Tanacetum*	
Tanacetum abrotanifolium (L.) Druce	Kurdestan, Baneh to Saghez; Rahiminejad & Dehghan; 15143 (1)
	Kurdestan, after Marivan, Chenareh toward Baneh, around Katicvn police station; Rahiminejad & Dehghan; 15143 (2)
Sect. *Balsamita*	
T. balsamita L.	Mazandaran, Golestanak; Naderi; 16621
	Lorestan, Azna; Rahiminejad & Dehghan; 16624
Sect. *Pyrethrellum*	
T. coccineum (Willd.) Grierson.	Mazandaran, pole Zanguleh; Naderi; 16722
Sect. *Xylanthemum*	
T. lingulatum (Boiss.) Bornm.	Isfahan, Meymeh, Muteh, Tkht-e Sorkh; Feyzi; 13215
	Isfahan, 30 km Esfahan to Shahreza, Gardan-e Lashotor; Nowroozi & Janighorban; 3855
	Isfahan, Morchekhort to Soh; Nowroozi & Shams; 11983
Sect.-	
T. partheniifolium (Willd.) Schuhz-Bip.	Isfahan, Semirom, Vanak, Dalankouh; Parishani; 14087
	Kohgilooyeh and Booyerahmad, Loodab; Aminimehr; 21890
T. persicum (Boiss.) Mozaffarian	Isfahan, 10 km Semirom to Vanak; Nowroozi; 4301
	Isfahan, Natanz, Karkas Mt.; Feyz & Shams; 10727
	Isfahan, Borujen, Kohyan, Tang-e Ghalandari; Nowroozi; 2541
Sect. *Xanthoglossa*	
T. pinnatum Boiss.	Isfahan, Fuladshahr to Juzdan, Tang-e Juzdan; Janighorban & Shams; 10791
	Fars, Shiraz, Kazeroum, Hosseinabad; Feyzi; 9069
	Isfahan, 63 km Kashan to Natanz; Nowroozi; 6661
T. polycephalum Schultz Bip.	Mazandaran, pole Zanguleh, Golestanak; Naderi; 16720
	Hamedan, Asadabad, Khan Gormaz Mountain; Kalvandi & Najafi; 16361
	Kohgilooyeh and Booyerahmad, Loodab, Nargesi, Mazeh region; Aminimehr; 21920
	Fars, Bavanat, Simakan; Feyzi & Shams; 12077
	Kohgilooyeh and Booyerahmad, between Sharekord and Farokhshahr; Nowroozi, 2783
	Isfahan, Khansar, Khansar Mt.; Feyzi; 6723
	Isfahan, Kashan, Ghamsar to Ghohrod; Shams; 10130
T. uniflorum (Fisch. & C.A. Mey. ex DC.) Schultz Bip.	Isfahan, Khansar, Golestankuh; Feyzi; 10041
	Isfahan, Meymeh, Laybid; Feyzi; 13213
	Isfahan, Semirom, Goortapacy; Nowroozi; 4322

Light Microscopy (LM) studies

Dried herbarium specimens were used for light microscopy observations. Leaf segments were initially placed in a solution of 70% ethanol and 30% acetic acid for 48–72 h. The samples were then rinsed with distilled water for 2–3 min and transferred to a 1:1 mixture of hydrogen peroxide and acetic acid. The material was heated at 80–100°C for approximately 10 min until bleaching was achieved. After clearing, thin layers from both the abaxial and adaxial leaf surfaces were carefully peeled, mounted on microscope slides, and examined to observe epidermal cell patterns and trichome occurrence. The prepared samples were examined and photographed using a light microscope at 40× magnification.

Scanning Electron Microscopy (SEM) studies

For SEM analysis, dried leaf segments were mounted on aluminum stubs using double-sided adhesive tape. The samples were sputter-coated with a thin layer (approximately 10 nm) of gold–palladium. Observations were carried out using a scanning electron microscope (SERON, AIS2300C) at the Materials Engineering Laboratory of Isfahan University of Technology, Isfahan, Iran. Micrographs were obtained at magnifications of 20×, 50×, and 100× to examine epidermal surface features and trichome structure.

Stomatal counting and analysis

Stomatal and epidermal cell counts were performed using a light microscope at 40× magnification with a calibrated scale of 30 µm. The stomatal index (SI) was calculated using the following formula: $SI = \frac{S}{(E+S)} \times 100$. Where S represents the number of stomata and E represents the number of non-stomatal epidermal cells. Image analysis and measurements were carried out using Digimizer software (Version 5.4.9).

RESULTS

In this study, nine species of the genus *Tanacetum* collected from different regions of Iran were examined using both quantitative and qualitative approaches. Quantitative data included stomatal counts and measurements of stomatal length and width, while qualitative observations focused on epidermal cell arrangement and stomatal structure. The results showed that epidermal cells could be grouped into two main types: regular (polygonal) and irregular. In addition, three stomatal types, namely anomocytic, paracytic, and anisocytic, were observed among the studied species, and the epidermal surface was either smooth or furrowed (Table 2). Light microscopy images of epidermal features and stomata are presented in Fig. 1. The scanning electron microscopy (SEM) images are shown in Fig. 2. SEM observations provided clearer details of epidermal surface features, including trichome morphology and stomatal structure. When the micromorphological observations were compared with the infrageneric classification presented in *Flora Iranica*, the examined taxa represented several sections, including sect. *Tanacetum* (*T. abrotanifolium*), sect. *Balsamita* (*T. balsamita*), sect. *Pyrethrellum* (*T. coccineum*), sect. *Xylanthemum* (*T. lingulatum*), and sect. *Xanthoglossa* (*T. pinnatum*, *T. polycephalum*, and *T. uniflorum*). In general, the qualitative epidermal characters were relatively similar among the examined sections, and no single character was found to clearly separate all sectional groups. Nevertheless, some quantitative differences were observed among taxa. The highest stomatal index and the largest stomata were recorded in *T. uniflorum* (sect. *Xanthoglossa*; SI=19.44; mean stomatal length=97.26 µm), whereas the lowest stomatal index was observed in *T. coccineum* (sect. *Pyrethrellum*; SI=7.40).

Table 2. Quantitative and qualitative traits, and micromorphological features of stomata and epidermal cells in the examined *Tanacetum* species.

Taxa	Stomatal type	Epidermal cell margin	Epidermal cell shape	Mean stomatal length (µm)	Mean stomatal width (µm)	Stomatal index (%)
Tanacetum abrotanifolium	Anomocytic	Furrowed	Irregular	72.71±9.36	48.47±5.97	14.16
T. balsamita	Anisocytic	Smooth	Polygonal	38.49±1.17	26.66±2.36	10.91
T. coccineum	Anomocytic	Furrowed	Irregular	62.20±3.29	57.67±6.27	7.40
T. lingulatum	Anomocytic	Furrowed	Irregular	37.25±3.15	26.52±2.02	10.02
T. parthenifolium	Paracytic	Furrowed	Irregular	46.61±7.92	35.36±4.3	14.21
T. persicum	Anomocytic	Furrowed	Irregular	64.05±5.51	57.70±8.37	13.50
T. pinnatum	Anomocytic	Furrowed	Irregular	88.77±8.98	71.72±20.02	10.52
T. polycephalum	Anomocytic	Furrowed	Irregular	48.81±4.06	37.05±2.22	14.54
T. uniflorum	Anomocytic	Furrowed	Irregular	97.26±8.89	54.21±4.30	19.44

Stomatal length ranged from 37.25 µm in *T. lingulatum* (sect. *Xylanthemum*) to 97.26 µm in *T. uniflorum*, and stomatal width varied from 26.52 µm in *T. lingulatum* to 71.72 µm in *T. pinnatum* (sect. *Xanthoglossa*). These observations suggest that epidermal micromorphological traits may be useful in distinguishing species, although their value for delimiting infrageneric groups appears to be limited. Micromorphological observations showed that trichomes were mainly distributed on the abaxial leaf surface in the studied *Tanacetum* species. Although trichomes were observed in all examined taxa (Fig. 2), different trichome forms were more clearly observed under LM in *T. polycephalum*, *T. uniflorum*, and *T. abrotanifolium* (Fig. 3). In the remaining species, trichomes were either sparsely distributed or showed limited morphological variation.

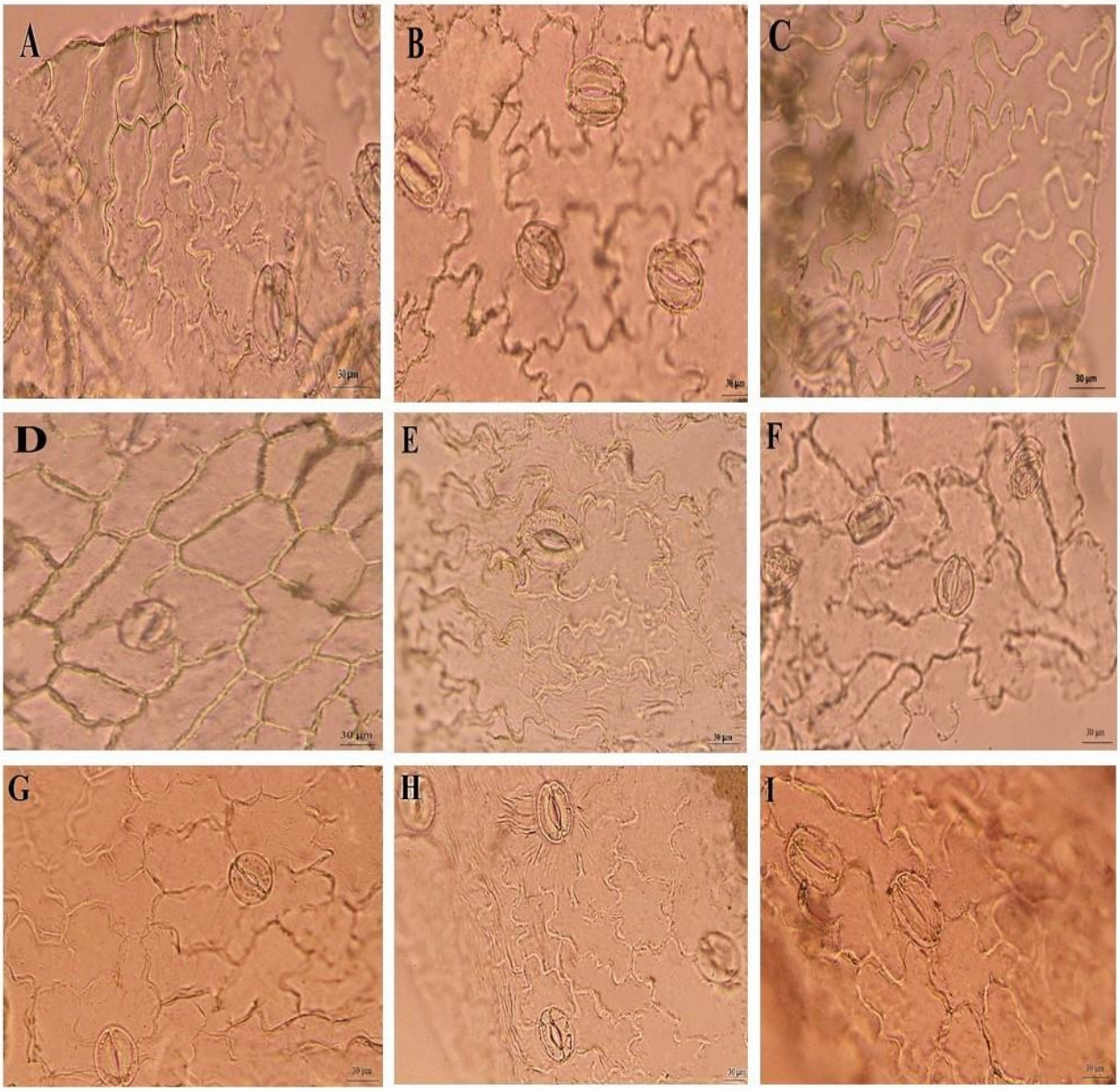

Fig. 1. Light microscopy images of leaf epidermis and stomata in *Tanacetum* species: A, *T. polycephalum;* B, *T. uniflorum;* C, *T. abrotanifolium;* D, *T. balsamita;* E, *T. coccineum;* F, *T. lingulatum;* G, *T. parthenifolium;* H, *T. persicum;* I, *T. pinnatum.* Scale bar=30 µm.

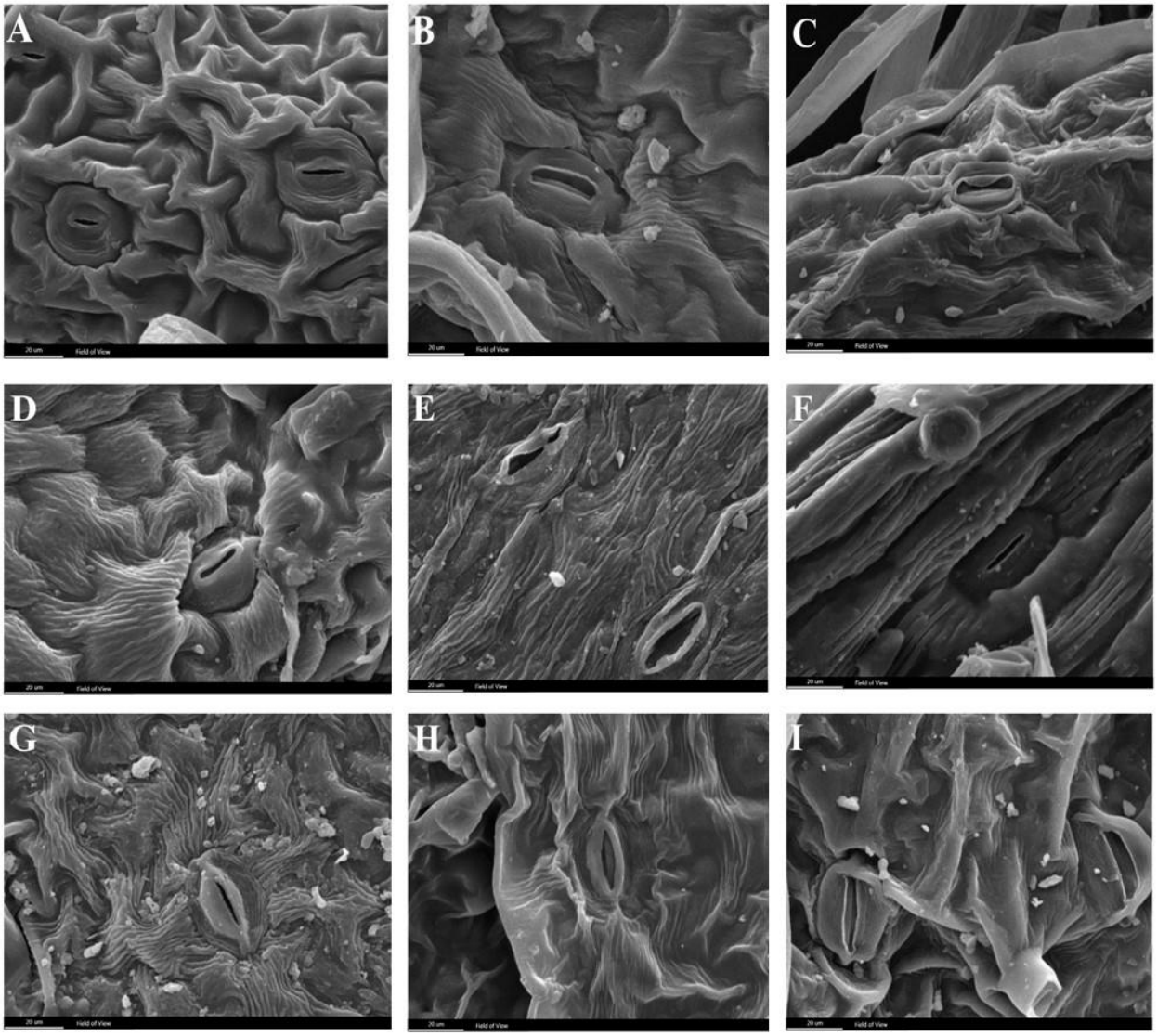

Fig. 2. Scanning electron microscopy (SEM) images of leaf epidermis and stomata in *Tanacetum* species: A, *T. polycephalum;* B, *T. uniflorum;* C, *T. abrotanifolium;* D, *T. balsamita;* E, *T. coccineum;* F, *T. lingulatum;* G, *T. parthenifolium;* H, *T. persicum;* I; *T. pinnatum.* Scale bar=20 μm.

DISCUSSION

Micromorphological features of the leaf epidermis in the examined *Tanacetum* species showed variation and were assessed for their taxonomic significance. The results indicate that traits such as stomatal type, epidermal cell shape, and trichome structure may provide useful information and can complement macromorphological characters in species delimitation. Three stomatal types, anomocytic, paracytic, and anisocytic, were identified among the studied species. Paracytic stomata were restricted to *T. parthenifolium*, anisocytic stomata were observed only in *T. balsamita,* and the remaining species showed the anomocytic type. This pattern is consistent with previous reports on stomatal diversity within Anthemideae (Chehregani & Mahanfar 2007; Karbalaei & al. 2021). The observed variation suggests that stomatal type may represent a potentially useful character for separating species groups. In particular, the distinction between *T. abrotanifolium* and *T. polycephalum* based on stomatal type highlights the usefulness of micromorphological traits where macromorphological differences are limited. Considerable variation was also observed in quantitative stomatal features.

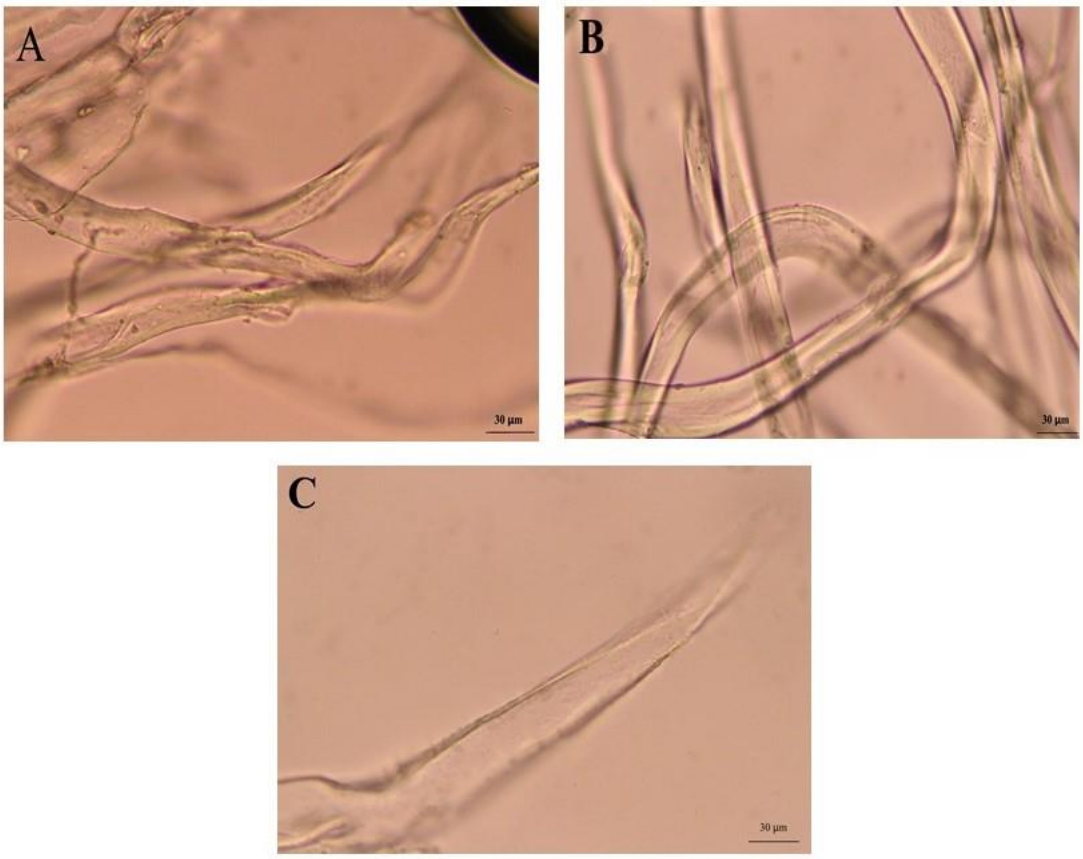

Fig. 3. Light microscopy images showing trichome variation in selected *Tanacetum* species: A, *T. polycephalum;* B, *T. uniflorum;* C, *T. abrotanifolium.* Scale bar=30 µm.

Stomatal length ranged from 37.25 µm in *T. lingulatum* to 97.26 µm in *T. uniflorum*, and the stomatal index varied from 7.40 in *T. coccineum* to 19.44 in *T. uniflorum*. Such variation has previously been associated with both genetic and environmental factors. In the present study, the examined specimens originated from different geographical regions and ecological conditions in Iran, which may have contributed to part of the observed variation in stomatal size and stomatal index. For example, differences observed between *T. uniflorum* and some other taxa may partly be related to differences in habitat conditions. However, further ecological and experimental studies would be required to evaluate this relationship more precisely. In general, the observed differences in stomatal index suggest that this character may have diagnostic value within the genus (Metcalfe & Chalk 1979). Epidermal cell morphology also showed variation among species. Two main types, polygonal (regular) and irregular, were distinguished. Most species were characterized by irregular cell outlines, whereas *T. balsamita* showed polygonal cells with a smooth surface. This combination of characters distinguished *T. balsamita* from the other studied taxa and was in agreement with previously reported morphological and chemical differences (Zandi & al. 2023). In contrast, the predominance of furrowed epidermal margins in other species may be associated with structural or ecological factors, although this would require further investigation. The use of SEM enabled detailed observation of epidermal surface features and trichome morphology. Some similarities were observed between the micromorphological characters identified in the present study and the molecular relationships reported by Sonboli & al. (2012). For example, *T. polycephalum* and *T. uniflorum*, which were placed close to each other in molecular analyses, also shared several epidermal features in the present study. In contrast, *T. balsamita* differed from the other examined taxa by its polygonal epidermal cells and smooth epidermal surface. Quantitative differences were also observed in *T. pinnatum*, particularly in stomatal measurements. Although these observations may indicate some

correspondence between epidermal traits and previously reported molecular relationships, the present data are not sufficient to establish direct phylogenetic interpretations. The molecular studies of Moradi Behjou & al. (2016) and Sonboli & al. (2012) provide a useful comparative framework for evaluating the micromorphological variation observed in *Tanacetum*. In the present study, some taxa that showed similarities in molecular analyses also shared certain epidermal features, whereas other taxa remained morphologically distinct. These observations support the view that micromorphological characters, together with other morphological and molecular evidence, may contribute to species delimitation and infrageneric studies in the genus. Trichome micromorphology has been regarded as a useful source of taxonomic information in Asteraceae (Ciccarelli & al. 2007). Napp-Zinn and Eble (1980) examined glandular and non-glandular trichomes in several genera of Anthemideae, and later studies also reported the occurrence of glandular trichomes in different vegetative and floral organs of the family (Ciccarelli & al. 2007). In the present study, two main trichome types, namely glandular and unicellular trichomes, were observed among the examined taxa. Similar trichome types have previously been reported in *Tanacetum* and other members of Asteraceae (Dere & Akcin 2017; Tekin & Kartal 2016; Giuliani & al. 2024). The observations obtained by SEM provided clearer visualization of trichome morphology and epidermal surface features, which may be useful in future taxonomic studies of the genus (Mandel & al. 2019; Criado-Ruiz & al. 2025). Overall, the micromorphological traits examined in this study showed variation among the studied *Tanacetum* species and may provide useful characters for species delimitation within the genus. The results suggest that the integration of micromorphological, macromorphological, and molecular data contributes to a better understanding of taxonomic relationships within the genus. Further studies, including a broader range of species and populations, are needed to better evaluate the taxonomic value of these characters.

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