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Pollen morphology of some *Tanacetum* L. (Asteraceae) taxa and its systematic value

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Abstract: Pollen grains of 24 *Tanacetum* taxa from Türkiye were examined by light (LM) and scanning electron microscopy (SEM). Detailed pollen morphological characteristics are provided for these taxa. According to the 24 analyzed taxa, *T. parthenifolium* has the smallest pollen grains, and *T. aurem* var. *aurem* possesses the largest ones. The basic shape of the pollen grains in most taxa is oblate-spheroidal. However, suboblate pollen grains are recorded for *T. balsamitoides*, *T. aurem* var. *aurem*, and *T. tomentellum.* The polar axis ranges from 16.56 to 26.14 µm, and the equatorial diameter ranges from 17.64 to 30.12 µm in this study. The grains are trizonocolporate, and exine sculpturing is echinate in all taxa. The ornamentations between spines are reticulate, microreticulate, perforate, microperforate, perforate-granulate, microperforate-granulate, rugulate-granulate, and rugulate-perforate. Pollen morphological characteristics of the taxa studied are compared and discussed on the basis of taxonomical concepts. The unweighted pair group approach with arithmetic mean was also employed to assess the morphological differentiation of the pollen, and four types were identified in the dendrogram created from the studied data using this method. In addition, principal component analysis confirms the dendrogram results. In some cases, the pollen characters are useful in distinguishing the taxa.

Key words: Asteraceae, cluster analysis, pollen morphology, *Tanacetum*, Türkiye

1. Introduction

Tanacetum L. is one of more than 110 genera of the Anthemideae tribe, which includes around 10% of the total Asteraceae genera and 15% of the species (Heywood and Humphries, 1977; Oberprieler et al., 2009; Olanj and Sonboli, 2021). The genus *Tanacetum* has over 160 species worldwide and consists primarily of perennial perennials with only a few annuals (Sonboli et al., 2012). Its species are found mostly in central and eastern Asia, Europe, the Mediterranean, and northern America (Korkmaz et al., 2015). *Tanacetum* is the tribe's third largest genus, after *Artemisia* L. and *Anthemis* L. (Sonboli et al., 2011). Several *Tanacetum* taxa are widely employed as sources of medications, food, or fodder and have considerable antibacterial, cytotoxic, neuroprotective, and antioxidant activities (Olanj et al., 2015; Yıldırım Doğan et al., 2019). They have traditionally been used to treat fevers, migraines, rheumatoid arthritis, stomach aches, toothaches, bug bites, infertility, and issues with menstruation and labor during childbirth (Kodak et al., 2017). In Türkiye, the *Tanacetum* is represented by 61 taxa, 27 of which are endemic (Arıtuluk et al., 2019).

Tanacetum has tremendous morphological diversity and convoluted taxonomic history, and *Tanacetum*'s phylogenetic position within the Anthemideae, generic delimitation, and infrageneric categorization have all been seen as problematic (Pulišová et al., 2021). It has been confused both taxonomically and nomenclatural with the *Pyrethrum* Medkus., *Balsamita* Mill., and *Chrysanthemum* L. in Anthemideae (Todorova and Evstatieva, 2001; Inceer et al., 2012).

A vast number of publications have proved that pollen morphology is an effective aid to plant taxonomy, and pollen morphology has offered a framework for understanding the systematic relationships among Asteraceae genera (Erdtman, 1952; Meo, 2009; Kodak et al., 2012). Asteraceae is a eurypalynous family, and the majority of its genera has zonocolporate pollen (Erdtman, 1952; Sachdeva and Malik, 1986). Also, Asteraceae pollen grains are essentially helianthoid, spherical or slightly flattened, tricolporate, and echinate (Wodehouse, 1935; Skvarla et al., 1977). The distinctive exine structure of the Asteraceae was established by Stix (1960) and Skvarla et al. (1977). In addition, Heywood and Humphries (1977)

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and Skvarla et al. (1977) published critical reviews of the pollen morphology in connection with the taxonomy and evolution of the Asteraceae. Also, it has been suggested that pollen shape, exine sculpture, and spine morphology were helpful for classification in Asteraceae (Shabestar et al., 2013; Ahmad et al., 2013). There are studies about the pollen morphologies of *Tanacetum* doing several researchers around the world and in Türkiye, and it has been shown that the genus *Tanacetum* has Anthemis-type echinate pollen, and they have different spins, which also have an important place in the classification (Wodehouse, 1926; Skvarla et al. 1977; Ramos and Mederos, 2008; Özmen et al., 2009; Kodak et al., 2012; Devrnja et al., 2012).

However, more studies are needed on the pollen morphology of the genus *Tanacetum*. Also, pollen studies of some of the taxa studied in this research were not generally found. The aim of the present study is to review and discuss the pollen morphological characteristics of 24 *Tanacetum* taxa collected from east of Anatolia (except for *T. macrophyllum* is collected from the Black Sea region) in order to determine micromorphological characteristics, assess their taxonomic value, and ultimately contribute to *Tanacetum* systematics by providing useful data using light microscopy (LM) and scanning electron microscopy.

2. Material and methods

2.1. Plant materials

The plants were collected from wild populations in Türkiye. Table 1 lists the collectors and locations of the specimens, and the distribution of taxa was given in the map (Figure 1). Plant specimens were stored in the Bitlis Eren University Herbarium (BEUH). In this study, pollen grains were obtained from anthers of flower buds of specimens held from different flowers (Table 1).

2.2. Pollen morphological analysis

Wodehouse's (1935) technique was used for LM studies to preserve the natural form of pollen grains. After removing oily substances with 70% ethyl alcohol, pollen grains were stained with glycerine jelly plus safranin on a slightly heated slide, and a coverslip was placed on top. These preparations were examined and measured using a light microscope. The images were captured using the Olympus CX-41 digital photomicrograph system. Polar axis (P), equatorial diameter (E), ratio of polar axis and equatorial diameter (P/E), colpus length (Clg), colpus width (Clt), porus length (Plg), porus width (Plt), exine thickness (ex) thickness of intine (in), mesocolpium (L), apocolpium (t), and operculum diameter (Op) were measured. At least 30–35 measurements were made for each character, and the medium and standard deviation were calculated (Tables 2 and 3). Morphological data for all taxa were presented (Tables 2 and 3). The terminology used generally follows that of Punt et al. (2007) and Hesse et al. (2018). P/E ratio was employed to determine the pollen shape according to the classification of Erdtman (1952): suboblate (0.88–0.75) and oblate-spheroidal (1.00–0.88).

Pollen grains were dried, mounted on stubs, and coated with gold using a sputter coaster for SEM studies. Polar and equatorial views, aperture, and ornamentation details of these pollen grains were photographed using a ZEISS Supra 55 Scanning Electron Microscope at the Central Research Laboratory (MERLAB), Yüzüncü Yıl University, Van.

2.3. Statistical analysis

Statistical studies were conducted on the pollen morphological characteristics obtained from 24 taxa (Tables 2 and 3). For the statistical analysis, 14 quantitative characters, the polar axis (P), equatorial diameter (E), porus length (Plg), porus width (Plt), colpus length (Clg), colpus width (Clt), exine thickness (Ex), intine thickness (In), apocolpium (t), mesocolpium (L), operculum diameter (Op), spine length (Sh), spine width (St), and distance between spines (Ds) were utilized. These characters were statistically analyzed using the MVSP software version 3.2 (MultiVariate Statistical Package) (Kovach, 1999). The UPGMA (unweighted pair group method with arithmetic mean) clustering method was used to group taxa with morphological similarities and establish taxa relationships. The Euclidean distance coefficient was employed in the cluster analysis to group 24 *Tanacetum* taxa. PCA (principal component analysis) was conducted on a dataset comprising 14 quantitative morphological characteristics of pollen obtained from 24 taxa of *Tanacetum*. The dataset needed to be standardized since the variables were already measured on the same scale. Prior to evaluating the data utilizing phylogenetic comparison methods, principal components analysis (PCA) is the most popular technique for reducing the dimensionality of the data collection (Uyeda et al., 2015). It achieves this by calculating many more minor variables (known as principle components) that represent the initial data set (Girgel, 2021).

3. Results

3.1. Size, shape, and symmetry

According to the LM, the pollen in all the taxa studied was trizonocolporate and operculate, shed as monads. They had the following dimensions after being treated with ethanol and embedded in glycerine jelly; polar axis (P) ranged from 16.56 µm (*T. parthenifolium*) to 26.14 µm (*T. aurem* var. *aurem*), and equatorial diameter (E) ranged from 17.64 µm (*T. parthenifolium*) to 30.12 µm (*T. aurem* var. *aurem*). The pollen size was the smallest in *T. parthenifolium*, and the largest in *T. aurem* var. *aurem*. The pollen outline was elliptical or circular in the equatorial view and circular in polar view (Table 2; Figures 2–5).

Table 1. The collection data of investigated *Tanacetum* specimens.

Taxa	**Locality**	**Voucher and specimen code**
T. abrotanifolium (L.) Druce	Bitlis: Tatvan-Hanelmalı village, Northern slopes, 1680 m, 14.08.2021.	M.Kürşat 7088
T. argenteum (Lam.) Willd. subsp. *argenteum*	Elazığ: Baskil, northern slopes of Hasan Mountain, Kayalık, 1650 m, 17.06.2021 **(Endemic).**	M.Kürşat 7082
T. aureum (Lam.) Greuter, M.V.Agab. & Wagenitz var. *aureum*	Van: Peli Mountain, Southern slopes, 2700 m, 14.06.2021.	M.Kürşat 7094
T. aureum var. *heimerlii* (Nábělek) Kandemir	Hakkâri: Cilo plateau, Avaspi glaciers, Mergan plateau, 2400 m, 28.06.2021	M.Kürşat 7090
T. aureum var. *oligocephalum* (Nábělek) Kandemir	Van: Güzeldere pass, Western slope, 2600 m, 04.07.20022.	M.Kürşat 8007
T. balsamitoides Sch.Bip.	Hakkâri: Berçalan plateau, between Büyükçel and Küçükçel, slopes, 2650 m, 28.08.2021.	M.Kürşat 7089
T. cadmeum subsp. *orientale* Grierson	Van: Gevaş-Artos Mountain, Southern slopes, 2500 m. 17.06.2021. **(Endemic).**	M.Kürşat 8000
T. canescens DC.	Muş: Kurtik mountain, Southern slopes, 2400 m, 05.06.2021.	M.Kürşat 7084
T. cappadocicum (DC.) Sch.Bip.	Elazığ: Baskil, Hasan Mountain, 2080 m, **(Endemic).**	M.Kürşat 8006
T. cilicium (Boiss.) Grierson	Bitlis: Tatvan, South-Eastern slopes of Kirkor Mountain, 2300 m, 23.07. 2021.	M. Kürşat 7081
T. densum subsp. *amani* Heywood	Elazığ: Baskil, northern slopes of Hasan mountain, 2000 m. 01.06.021. **(Endemic).**	M.Kürşat 7091
T. densum subsp. *laxum* Grierson	Elazığ: Baskil, northern slopes of Hasan Mountain, 1650 m, 01.06.021. **(Endemic).**	M.Kürşat 7083
T. heterotoum (Bornm.) Grierson	Elazığ: Baskil, Yukarı Kuluşağı village, 28.06.2022 **(Endemic).**	M.Kürşat 8004
T. kotschyi (Boiss.) Grierson	Van: Gevaş, Artos Mountain, Southern slopes, 2500 m, 15.06.2021.	M.Kürşat 7096
T. macrophyllum (Waldst. & Kit.) Sch.Bip.	Artvin: Murgul-Damar, Kabaca plateau, 2000 m, 30.06.2021.	M.Kürşat 7085
T. nitens (Boiss. & Noë) Grierson	Van: Peli Mountain, Southern slopes, 2700 m, 14.06.2021 **(Endemic).**	M.Kürşat 7093
T. parthenifolium (Willd.) Sch.Bip.	Bitlis: Bitlis-Tatvan road, slopes, 1650 m, 28.09.2021.	M.Kürşat 7099
T. parthenium (L.) Sch.Bip.	Tunceli: Pülümür-Erzincan road, 2 km after the Ardıçlı village junction, waterside, 1575 m, 23.06.2021.	M.Kürşat 7087
T. polycephalum subsp. *argyrophyllum* (K.Koch) Podlech	Bitlis: Tatvan, South-Eastern slopes of Kirkor Mountain, Rocky, 2415 m, 23.07.2021.	M.Kürşat 7080
T. tabrisianum (Boiss.) Sosn. & Takht.	Van: Güzeldere pass, Western slope, 2600 m, 04.07.20022.	M.Kürşat 8010
T. tomentellum (Boiss.) Grierson	Bitlis: Kambos Mountain, Northern slopes, 1750 m, 07.09.2021.	M.Kürşat 7098
T. uniflorum (Fisch. & C.A.Mey.) Sch.Bip.	Van: Güzeldere pass, Western slope, 2600 m, 04.07.20022.	M.Kürşat 8008
T. vulgare L.	Tunceli: Ovacik, Munzur cellars, 1400 m, 24.06.2021.	M.Kürşat 7086
T. zahlbruckneri (Náb.) Grierson	Van: Peli Mountain, Southern slopes, 2700 m, 14.06.2021 **(Endemic).**	M.Kürşat 7095

3.2. Apertures

The colpus had distinct and regular margins, as well as acute ends. The size of colpus varied from 11.89 µm (*T. parthenifolium*) to 19.27 µm (*T. aurem* var. *aurem*) in length and from 5.02 µm (*T. parthenifolium*) to 9.72 µm (*T. kotschyi*) in width. Porus could be circular or lalongate in shape. Porus size ranged from 5.13 µm (*T. parthenifolium*) to 10.81 µm (*T. kotschyi*) in length and from 5.02 µm (*T. parthenifolium*) to 9.48 µm (*T. kotschyi*) in width. Mesocolpium varied between 17.13 µm (*T. parthenifolium*) and 29.47 µm (*T. aurem* var. *aurem*). Operculum diameter varied between 2.35 µm (*T. parthenifolium*) and 7.14 µm (*T. abrotanifolium*). Apocolpium varied between 4.69 µm (*T. parthenifolium*) and 8.16 µm (*T. argentum* subsp. *argentum*) (Table 2; Figures 2–5).

Figure 1. Location map showing in red the regions where plant specimens were collected (modified from Ekinci et al., 2020).

3.3. Exine, intine, spine, and ornamentation

Exine sculpturing was echinate in all investigated taxa, and in SEM, the ornamentations between spines were reticulate, microreticulate, perforate, microperforate, perforate-granulate, microperforate-granulate, rugulate-granulate, and rugulate-perforate (Table 3). Ectexine was thicker than endexine without costae and cavea. The exine thickness varied between 1.98 µm (*T. cadmeum* subsp. *orientale*) and 2.39 µm (*T. aurem* var. *aurem*). The intine thickness varied between 0.85 µm (*T. parthenifolium*) and 1.17 µm (*T. aurem var. aurem*) (Tables 2 and 3; Figures 4 and 5).

The spines were generally conical with a broadened base and gradually tapered into pointed tips. Spine length ranged from 2.10 µm (*T. cadmeum* subsp. *orientale*) to 4.70 µm (*T. nitens*), and spine width ranged from 3.0 µm (*T. densum* subsp. *laxum*) to 6.0 µm (*T. canascens*). Perforations at the base of the spines were found to be differently seriate in investigated taxa. Most species had 1–3 seriate perforations, and some had 1–2 or rarely 1–4 seriate perforations. The number of spines per 100 µm^2 was 6–27 in all studied taxa. The number of spines was highest for *T. kotschyi* (27), and lowest for *T. cadmeum* subsp. *orientale* (6). Distance between spines ranged from 2.7 µm (*T. cadmeum* subsp. *orientale)* to 7.3 µm (*T. nites*) in all of the studied taxa (Table 3; Figures 4 and 5).

3.4. Statistical analysis results of the palynological characteristics

Cluster analysis (UPGMA) was used to evaluate the similarity grouping of the 24 taxa based on the 14 morphological features measured. A dendrogram was used to display the results (Figure 6). The UPGMA indicated four classes based on the clustering results. According to the UPGMA dendrogram, there were four categories of taxa: types 1 and 2 had two subtypes, type 3 had fourteen subtypes, and type 4 had other taxa (Figure 6).

Table 4 displayed the PCA findings, including both axes' eigenvalues, variance percentages, and cumulative variance percentages. The PCA results explained 89.036% of the variation. Figure 7A depicted a PCA biplot of the sample scores of individual spectra and loading (eigenvectors) for *Tanacetum* taxa dispersion. The first two principal component axes were significant, accounting for 82.374% and 6.662% of the variance, respectively (Table 4). According to the results of variable loadings in Table 4, it was demonstrated that the polar axis, equatorial diameter, and mesocolpium had an inverse function in positioning type 1 (*T. parthenifolium* and *T. parthenium*). Type 1 comprised the taxa with the smallest pollen size, and the pollen size significantly impacted type 2 classification (*T. aurem* var. *aurem* and *T. cappadocicum*). Type 2 taxa were those with the highest pollen size.

Similarly, the polar axis, equatorial diameter, and mesocolpium were crucial in developing type 3 and type 4 (Figures 7A and 7B). From Figures 6 and 7, it was evident that the types obtained through UPGMA correspond to the types obtained through PCA analysis. Therefore, the UPGMA and PCA results supported each other.

4. Discussion

This study showed that the pollen morphology is a systematically significant character for the studied

Table 2. Summary of pollen morphological data for the *Tanecetum* taxa (µm) (mean ± standard deviation).

Taxa	P	E	P/E	Plg	Plt	Clg	Clt	Ex	In	t	L	Op.
T. abrotanifolium	25.50 ± 1.46	26.00 ± 1.32	O-s	10.02 ± 0.83	8.16 ± 0.68	18.36 ± 1.04	8.16 ± 0.68	2.04 ± 0.24	1.02 ± 0.18	7.14 ± 0.66	24.48 ± 1.27	7.14 ± 1.03
T. argentum subsp. *argentum*	23.46 ± 1.10	26.52 ± 1.06	O-s	9.18 ± 0.66	7.14 ± 0.78	16.32 ± 0.93	7.14 ± 0.78	2.04 ± 0.20	1.02 ± 0.17	8.16 ± 0.58	22.44 ± 1.11	6.12 ± 0.99
T. aurem var. *aurem*	26.14 ± 1.03	30.12 ± 0.86	S	9.69 ± 0.51	8.44 ± 0.61	19.27 ± 0.96	8.44 ± 0.61	2.39 ± 0.25	1.17 ± 0.21	7.24 ± 0.76	29.47 ± 1.12	6.48 ± 0.96
T. aurem var. *heimerlii*	22.50 ± 1.01	25.56 ± 1.17	O-s	8.22 ± 0.94	7.68 ± 0.86	16.93 ± 0.93	7.68 ± 0.86	2.04 ± 0.16	1.04 ± 0.17	6.79 ± 0.80	25.35 ± 1.10	4.85 ± 1.16
T. aureum var. *oligocephalum*	22.90 ± 0.95	24.23 ± 1.19	O-s	7.96 ± 0.55	7.23 ± 1.10	15.76 ± 0.97	7.23 ± 0.55	2.16 ± 0.23	1.10 ± 0.21	6.40 ± 0.93	24.46 ± 0.97	4.06 ± 1.11
T. balsamitoides	22.90 ± 1.35	26.34 ± 1.17	S	8.87 ± 0.87	8.01 ± 0.82	17.67 ± 1.02	8.01 ± 0.82	2.12 ± 0.22	0.98 ± 0.12	6.15 ± 0.61	25.12 ± 1.24	5.20 ± 1.21
T. cadmeum subsp. *orientale*	20.20 ± 1.09	22.20 ± 1.21	O-s	6.04 ± 0.93	5.83 ± 1.05	13.80 ± 0.99	5.83 ± 1.05	1.98 ± 0.16	0.89 ± 0.25	5.33 ± 0.80	21.83 ± 1.05	3.80 ± 1.21
T. canascens	20.02 ± 0.85	22.13 ± 0.83	O-s	6.52 ± 0.85	6.07 ± 0.55	13.87 ± 0.81	6.07 ± 0.55	2.02 ± 0.14	1.00 ± 0.15	5.74 ± 0.80	20.80 ± 0.93	3.63 ± 0.93
T. cappadocicum	26.10 ± 1.29	28.06 ± 1.28	O-s	8.13 ± 0.97	7.26 ± 0.82	18.66 ± 0.95	7.26 ± 0.82	2.34 ± 0.17	1.15 ± 0.21	7.36 ± 0.88	28.80 ± 0.96	4.56 ± 0.85
T. cilicium	20.29 ± 0.92	22.26 ± 1.02	O-s	7.88 ± 0.82	6.96 ± 0.64	14.95 ± 0.80	6.96 ± 0.64	2.09 ± 0.21	1.06 ± 0.21	5.97 ± 0.77	20.80 ± 0.85	4.44 ± 0.92
T. densum subsp. *amani*	22.29 ± 1.10	24.00 ± 0.86	O-s	8.08 ± 0.58	7.14 ± 0.58	16.62 ± 0.87	7.14 ± 0.58	2.05 ± 0.11	1.00 ± 0.10	7.03 ± 0.66	23.18 ± 1.14	4.47 ± 0.93
T. densum subsp. *laxum*	21.04 ± 0.89	22.70 ± 0.90	O-s	8.46 ± 0.83	7.78 ± 0.80	16.04 ± 0.78	7.78 ± 0.81	2.12 ± 0.20	1.09 ± 0.18	6.32 ± 0.84	21.78 ± 0.89	5.74 ± 1.10
T. heterotoum	22.60 ± 1.27	24.33 ± 1.12	O-s	6.66 ± 1.12	5.83 ± 0.79	16.90 ± 1.15	5.83 ± 0.79	2.11 ± 0.22	1.09 ± 0.18	6.46 ± 0.86	22.53 ± 0.86	4.50 ± 1.00
T. kotschyi	24.17 ± 1.12	27.50 ± 1.37	O-s	10.81 ± 0.77	9.48 ± 0.73	17.91 ± 0.86	9.72 ± 0.73	2.11 ± 0.22	1.02 ± 0.16	6.93 ± 1.03	24.40 ± 1.24	4.50 ± 1.20
T. macrophyllum	20.15 ± 0.93	21.55 ± 1.07	O-s	6.38 ± 0.82	6.15 ± 0.80	14.41 ± 0.86	6.15 ± 0.80	2.06 ± 0.21	1.00 ± 0.12	5.87 ± 0.73	20.97 ± 0.93	3.16 ± 0.76
T. nitens	24.64 ± 1.11	26.62 ± 1.15	O-s	7.75 ± 0.85	7.34 ± 0.76	17.70 ± 0.96	7.34 ± 0.76	2.01 ± 0.16	0.97 ± 0.16	6.69 ± 0.77	26.07 ± 1.00	4.21 ± 1.19
T. parthenifolium	16.56 ± 0.85	17.64 ± 0.85	O-s	5.13 ± 0.55	5.02 ± 0.74	11.89 ± 0.76	5.02 ± 0.74	2.00 ± 0.15	0.85 ± 0.20	4.69 ± 0.85	17.13 ± 1.16	2.35 ± 0.98
T. parthenium	17.13 ± 1.21	19.07 ± 1.18	O-s	5.71 ± 0.93	5.50 ± 0.85	12.44 ± 0.96	5.50 ± 0.85	2.04 ± 0.12	0.99 ± 0.14	4.72 ± 0.85	19.03 ± 1.12	3.36 ± 0.91
T. polycephalum subsp. *argyrophyllum*	23.36 ± 1.18	24.78 ± 1.18	O-s	8.42 ± 0.82	7.50 ± 0.85	16.86 ± 0.94	7.50 ± 0.85	2.11 ± 0.20	1.08 ± 0.20	6.35 ± 0.86	23.21 ± 1.04	4.82 ± 1.17
T. tabrisianum	23.93 ± 1.11	25.63 ± 1.15	O-s	7.66 ± 0.88	6.76 ± 0.77	15.80 ± 1.12	6.76 ± 0.77	2.26 ± 0.26	1.16 ± 0.22	6.46 ± 1.07	24.80 ± 0.99	3.80 ± 1.06
T. tomentellum	21.88 ± 0.86	25.09 ± 0.93	S	8.57 ± 0.63	8.01 ± 0.68	17.09 ± 1.20	8.01 ± 0.68	2.14 ± 0.21	1.07 ± 0.20	6.66 ± 0.90	23.50 ± 1.16	5.20 ± 0.93
T. uniflorum	22.50 ± 1.27	24.56 ± 1.10	O-s	7.40 ± 0.94	6.53 ± 0.88	15.96 ± 1.21	6.53 ± 1.00	2.23 ± 0.20	1.05 ± 0.28	6.50 ± 0.86	23.53 ± 1.04	3.86 ± 1.13
T. vulgare	20.83 ± 0.86	22.95 ± 0.82	O-s	5.59 ± 0.93	7.44 ± 0.91	15.60 ± 0.80	7.44 ± 0.91	2.09 ± 0.22	1.00 ± 0.15	6.32 ± 0.92	21.78 ± 0.80	5.53 ± 0.97
T. zahlbruckneri	24.17 ± 0.87	26.01 ± 1.10	O-s	8.42 ± 0.78	7.47 ± 0.80	16.96 ± 1.10	7.47 ± 0.80	2.15 ± 0.22	1.08 ± 0.22	7.24 ± 0.66	26.11 ± 1.10	4.89 ± 1.10

Nomenclature: Polar axis (**P**), equatorial diameter (**E**), pollen shape (**P/E**), porus length (**Plg**), porus width (**Plt**), colpus length (**Clg**), colpus width (**Clt**), exine thickness (**Ex**), mesocolpium (**L**), apocolpium **(t),** intine thickness (**In**), oblate-spheroidal (**O-s),** suboblate (**S**), operculum diameter **(Op)**.

Table 3. SEM analysis results of *Tanacetum* taxa (µm) (mean ± standard deviation).

Taxa	Spine length (Sh)	Spine width (St)	The number of spine per 100 µm²	Distance between spines	Perforation number at the base of the spines	Seriate perforations in the base of the spines	Ornamentation	Ornamentation of between spines
T. abrotanifolium	3.3 ± 0.08	4.1 ± 0.11	9 ± 1.29	5.4 ± 0.25	21 ± 2.21	1-3 irregular	Echinate	Microreticulate
T. argentum subsp. *argentum*	4.5 ± 0.17	5.0 ± 0.23	12 ± 2.05	5.5 ± 0.19	20 ± 1.83	1-2 irregular	Echinate	Rugulate-perforate
T. aurem var. *aurem*	2.9 ± 0.06	4.1 ± 0.17	10 ± 1.33	5.4 ± 0.22	27 ± 2.06	1-2 irregular	Echinate	Rugulate-perforate
T. aurem var. *heimerlii*	2.9 ± 0.21	3.8 ± 0.13	11 ± 1.35	5.0 ± 0.16	28 ± 1.86	1-3 irregular	Echinate	Perforate
T. aureum var. *oligocephalum*	2.8 ± 0.16	3.6 ± 0.09	12 ± 1.03	5.0 ± 0.37	29 ± 2.10	1-3 irregular	Echinate	Perforate
T. balsamitoides	3.6 ± 0.14	4.0 ± 0.14	13 ± 1.10	5.4 ± 0.15	26 ± 1.69	1-2 irregular	Echinate	Microperforate-granulate
T. cadmeum subsp. *orientale*	2.1 ± 0.15	3.1 ± 0.09	6 ± 1.27	2.7 ± 0.14	21 ± 2.01	1-3 irregular	Echinate	Perforate
T. canascens	4.5 ± 0.09	6.0 ± 0.23	10 ± 1.14	7.0 ± 0.19	26 ± 1.99	1-3 irregular	Echinate	Rugulate-perforate
T. cappadocicum	3.5 ± 0.11	4.9 ± 0.18	7 ± 1.38	5.7 ± 0.16	28 ± 2.11	1-4 irregular	Echinate	Rugulate-perforate
T. cilicium	4.3 ± 0.19	5.0 ± 0.13	10 ± 2.01	5.5 ± 0.24	18 ± 1.65	1-3 irregular	Echinate	Microperforate-granulate
T. densum subsp. *amani*	3.6 ± 0.11	3.6 ± 0.09	21 ± 2.06	4.2 ± 0.19	20 ± 2.19	1-2 irregular	Echinate	Microreticulate
T. densum subsp. *laxum*	2.5 ± 0.09	3.0 ± 0.08	10 ± 1.38	3.9 ± 0.22	22 ± 1.20	1-3 irregular	Echinate	Perforate
T. heterotoum	3.2 ± 0.16	3.8 ± 0.07	19 ± 2.11	6.1 ± 0.33	23 ± 2.10	1-3 irregular	Echinate	Rugulate-granulate
T. kotschyi	3.3 ± 0.11	3.8 ± 0.05	27 ± 1.47	5.5 ± 0.15	22 ± 2.26	1-3 irregular	Echinate	Perforate-granulate
T. macrophyllum	3.6 ± 0.17	5.0 ± 0.12	10 ± 1.11	7.2 ± 0.14	23 ± 1.83	1-2 irregular	Echinate	Microreticulate
T. nitens	4.7 ± 0.17	4.2 ± 0.17	12 ± 1.33	7.3 ± 0.22	25 ± 2.11	1-3 irregular	Echinate	Microperforate
T. parthenifolium	2.9 ± 0.06	3.3 ± 0.11	13 ± 1.18	4.1 ± 0.31	26 ± 2.34	1-3 irregular	Echinate	Perforate
T. parthenium	3.0 ± 0.11	4.2 ± 0.12	11 ± 1.20	3.4 ± 0.22	18 ± 1.87	1-4 irregular	Echinate	Perforate
T. polycephalum subsp. *argyrophyllum*	3.3 ± 0.15	5.0 ± 0.16	17 ± 1.41	6.1 ± 0.55	21 ± 2.23	1-3 irregular	Echinate	Rugulate-granulate
T. tabrisianum	3.6 ± 0.09	4.9 ± 0.10	15 ± 1.55	6.3 ± 0.26	22 ± 1.43	1-3 irregular	Echinate	Rugulate-perforate
T. tomentellum	3.4 ± 0.06	3.8 ± 0.17	9 ± 0.60	5.7 ± 0.18	26 ± 1.70	1-3 irregular	Echinate	Reticulate
T. uniflorum	3.1 ± 0.08	4.2 ± 0.16	11 ± 1.07	6.2 ± 0.23	33 ± 1.54	1-2 irregular	Echinate	Microreticulate
T. vulgare	3.6 ± 0.06	4.5 ± 0.09	14 ± 1.37	5.4 ± 0.20	20 ± 1.55	1-3 irregular	Echinate	Microperforate-granulate
T. zahlbruckneri	4.0 ± 0.11	5.5 ± 0.11	7 ± 1.80	6.5 ± 0.45	23 ± 1.51	1-3 irregular	Echinate	Reticulate

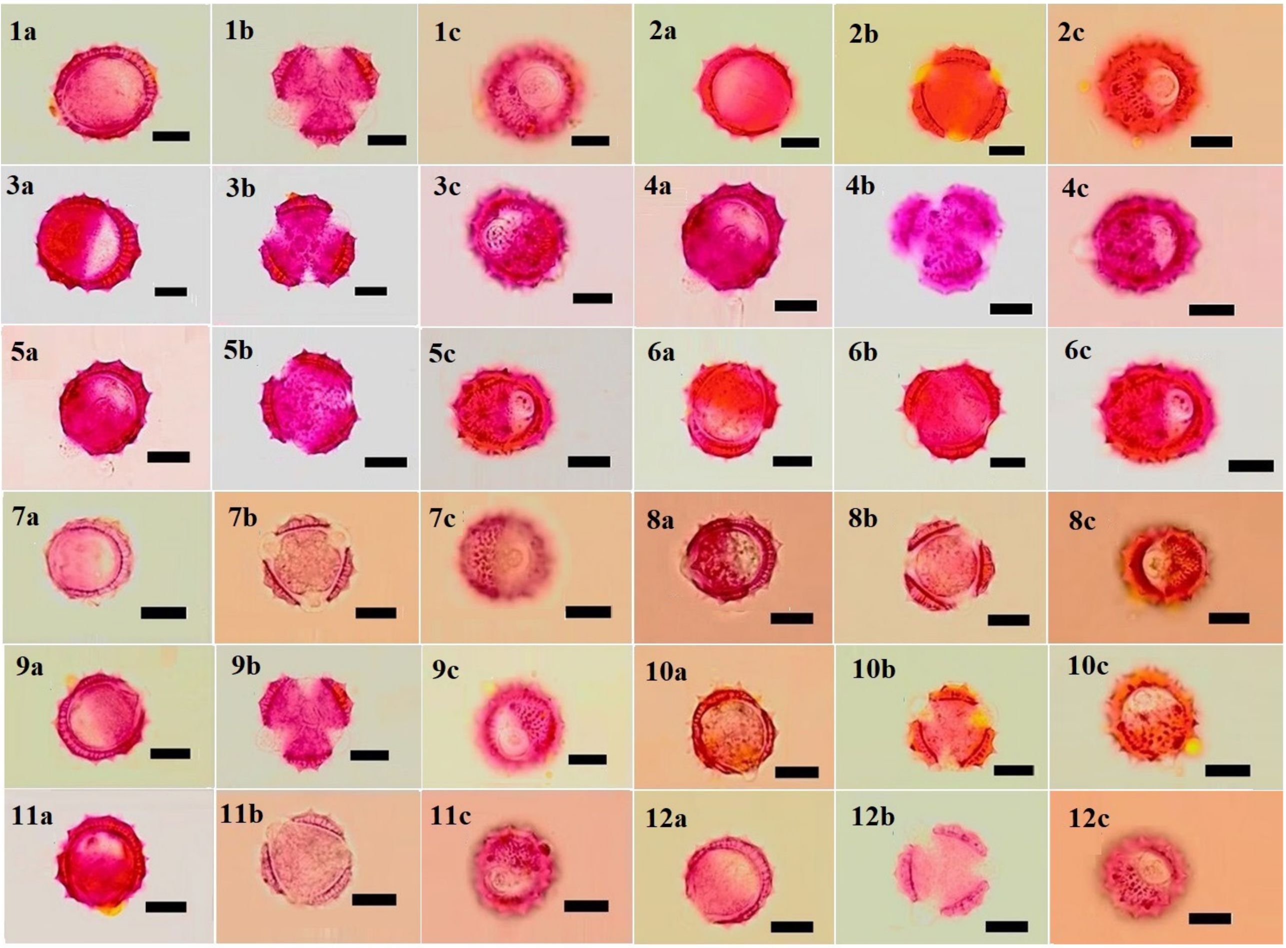

Figure 2. *Tanacetum* by light microscopy; *T. abrotanifolium* **(1a-1b-1c)**, *T. argentum* subsp. *argentum* **(2a-2b-2c)**, *T. aurem* var. *aurem* **(3a-3b-3c),** *T. aurem* var. *heimerlii* **(4a-4b-4c)**, *T. aureum* var. *oligocephalum* **(5a-5b-5c)**, *T. balsamitoides* **(6a-6b-6c)**, *T. cadmeum* subsp. *orientale* **(7a-7b-7c)**, *T. canascens* **(8a-8b-8c)**, *T. cappadocicum* **(9a-9b-9c)**, *T. cilicium* **(10a-10b-10c)**, *T. densum* subsp. *amani* **(11a-11b-11c)**, *T. densum* subsp. *laxum* **(12a-12b-12c) (a: equatorial view, b: polar view, c: apertural view) (scale bar 10 μm).**

Tanacetum taxa and for comparison with other genera in the Asteraceae. Pollen grains are similar generally concerning the type of aperture (tricolporate). The pollen shape of *Tanacetum* taxa investigated in our study was oblate-spheroidal, with only a few exceptions being suboblate. Also, Ramos and Mederos (2008) found that the pollen shapes of *Tanacetum* are mostly oblate-spheroidal, with some suboblate, spheroidal, or prolate-spheroidal variations. In addition, Inceoglu and Karamustafa (1977) found similar results to our study in which they determined that the pollen shape of *T. parthenium* is oblate-spheroidal.

Exine features have been reported to be important in systematic and phylogenetic classifications in Asteraceae, and *Tanacetum* is characterized by echinate pollen grains (Anthemis-type) (Skvarla et al., 1977). Further, it has been revealed that exine thickness varies substantially in the Asteraceae and that practically every species has a varied exine thickness (Ahmad et al., 2013). The ornamentation of 24 *Tanacetum* taxa studied in our study was found to be echinate, and the thickness of the intine has also been measured to range from 0.85 μm to 1.17 μm while the exine thickness ranged between 1.98 μm and 2.39 μm. According to Ahmad et al. (2013), the exine thickness of *Tanacetum* taxa ranges between 4.5 μm and 6.5 μm, and ornamentation is echinate. Also, according to Al-Fredan's (2019) pollen study on Asteraceae, including *T. santolinoides*, the pollen grains were echinate and oblate-spheroidal, with an exine thickness of 3.7–5.1 μm.

Asteraceae were thought to be distinguished by ornamentation between the spines since the arrangement, form, length, and size of the spines and also pollen size, spine length, as well as the morphological characteristics, vary more or less (Wodehouse, 1926; Clark et al., 1980; Kodak et al., 2012). Pollen grains from the genus

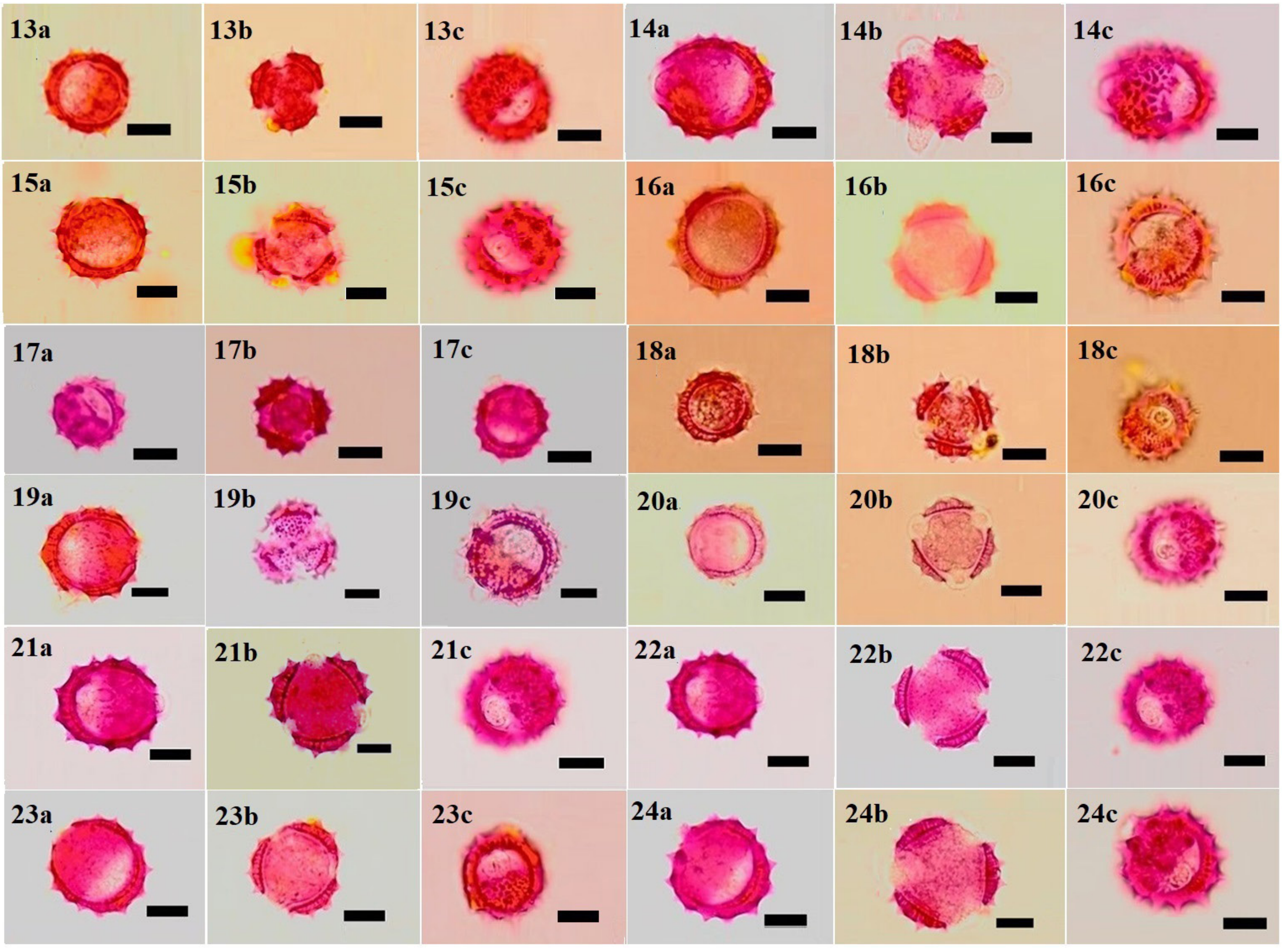

Figure 3. *Tanacetum* by light microscopy; *T. heterotoum* **(13a-13b-13c)**, *T. kotschyi* **(14a-14b-14c)**, *T. macrophyllum* **(15a-15b-15c)**, *T. nitens* **(16a-16b-16c)**, *T. parthenifolium* **(17a-17b-17c)**, *T. parthenium* **(18a-18b-18c)**, *T. polycephalum* subsp. *argyrophyllum* **(19a-19b-19c)**, *T. tabrisianum* **(20a-20b-20c)**, *T. tomentellum* **(21a-21b-21c)**, *T. uniflorum* **(22a-22b-22c)**, *T. vulgare* **(23a-23b-23c)**, *T. zahlbruckneri* **(24a-24b-24c)**, **(a: equatorial view, b: polar view, c: apertural view) (scale bar 10 µm).**

Tanacetum also have distinct spines and can be made to serve in their classification, according to Wodehouse (1926). Also, the pollen spine has evolutionary significance and specific and generic significance in the classification of Asteraceae (Zafar et al., 2007). In the analyzed taxa, perforations at the base of the spines are variable in our study. Kodak et al. (2012) demonstrated that the base of the spines in almost all *Tanacetum* taxa studied has 3 or 4 irregular seriate perforations with larger holes which are often found distally. In addition, according to a study conducted by Kodak et al. (2012), the ornamentation between spines in eight *Tanacetum* taxa was found to be granulated, reticulate (*T. tomentallum*), or rugulate-granulate (including *T. argentum*). In our study, however, the ornamentation between spines was different, along with what Kodak et al. (2012) have discovered.

Our study showed that *T. nitens* had the most extended spine length and *T. canascens* had the largest spine width, while *T. cadmeum* subsp. *orientale* had the smallest spine length and width. Several studies agree with the spin length results of our study (Ramos and Mederos, 2008; Devrnja et al., 2012). Similar to the findings of Özmen et al. (2009), our study demonstrated that the spines of the taxa under study are longer than those of many other Asteraceae taxa (Diez et al., 1994; Vega and Dematteis, 2011; Hayat et al., 2023). Furthermore, our research discovered that *T. cadmeum* subsp. *orientale* and *T. cappadacicum* have the fewest spines per 100 µm^2 while *T. kotschyi* has the most spines per 100 µm^2. As well, *T. densum* subsp. *laxum* has fewer spines per 100 µm^2 than *T. densum* subsp. *amani*. Our results concluded that the spine characters, including spine length, width, the number of spines, perforation

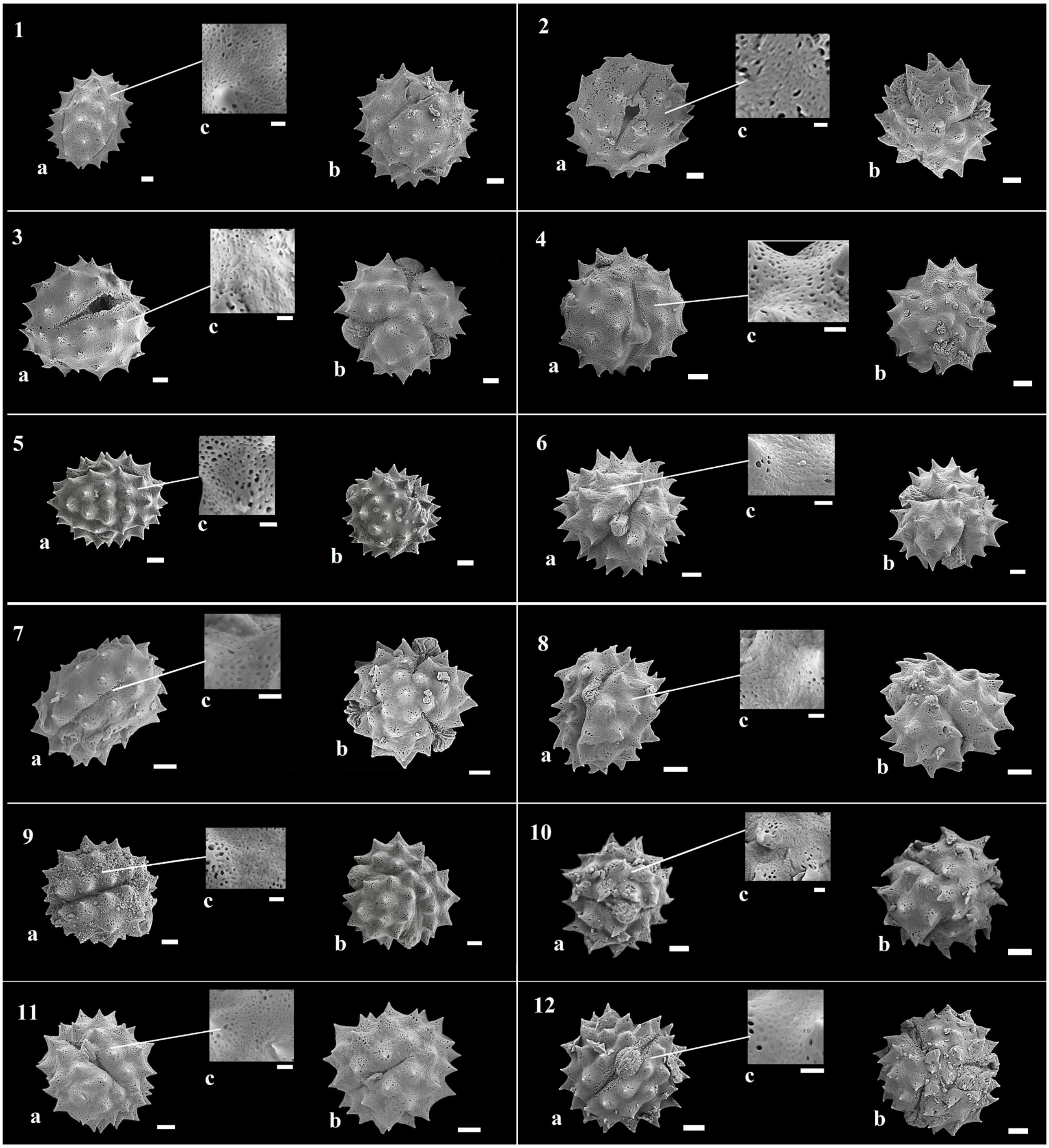

Figure 4. *Tanacetum* by scanning electron microscopy; *T. abrotanifolium* (**1a-1b-1c**), *T. argentum* subsp. *argentum* (**2a-2b-2c**), *T. aurem* var. *aurem* (**3a-3b-3c**), *T. aurem* var. *heimerlii* (**4a-4b-4c**), *T. aureum* var. *oligocephalum* (**5a-5b-5c**), *T. balsamitoides* (**6a-6b-6c**), *T. cadmeum* subsp. *orientale* (**7a-7b-7c**), *T. canascens* (**8a-8b-8c**), *T. cappadocicum* (**9a-9b-9c**), *T. cilicium* (**10a-10b-10c**), *T. densum* subsp. *amani* (**11a-11b-11c**), *T. densum* subsp. *laxum* (**12a-12b-12c**) **(a: polar view, b: equatorial view, c; ornamentation) (a, b: scale bar 4 µm, c: scale bar 1 µm).**

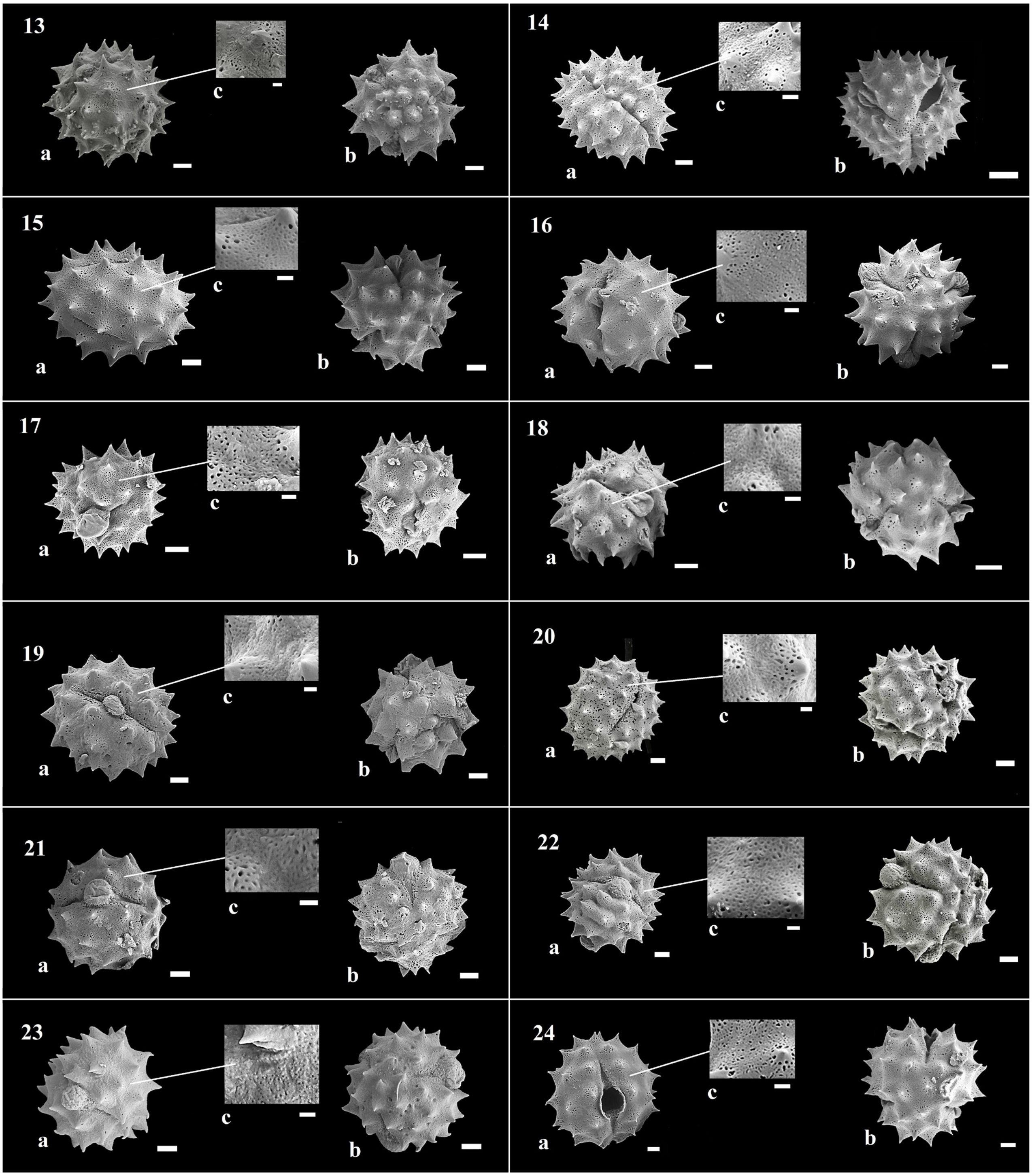

Figure 5. *Tanacetum* by scanning electron microscopy; *T. heterotoum* **(13a-13b-13c)**, *T. kotschyi* **(14a-14b-14c)**, *T. macrophyllum* **(15a-15b-15c)**, *T. nitens* **(16a-16b-16c)**, *T. parthenifolium* **(17a-17b-17c)**, *T. parthenium* **(18a18-b-18c)**, *T. polycephalum* subsp. *argyrophyllum* **(19a-19b-19c)**, *T. tabrisianum* **(20a-20b-20c)**, *T. tomentellum* **(21a-21b-21c)**, *T. uniflorum* **(22a-22b-22c)**, *T. vulgare* **(23a-23b-23c)**, *T. zahlbruckneri* **(24a-24b-24c)**, **(a: polar view, b: equatorial view, c; ornamentation) (a, b: scale bar 4 µm, c: scale bar 1 µm).**

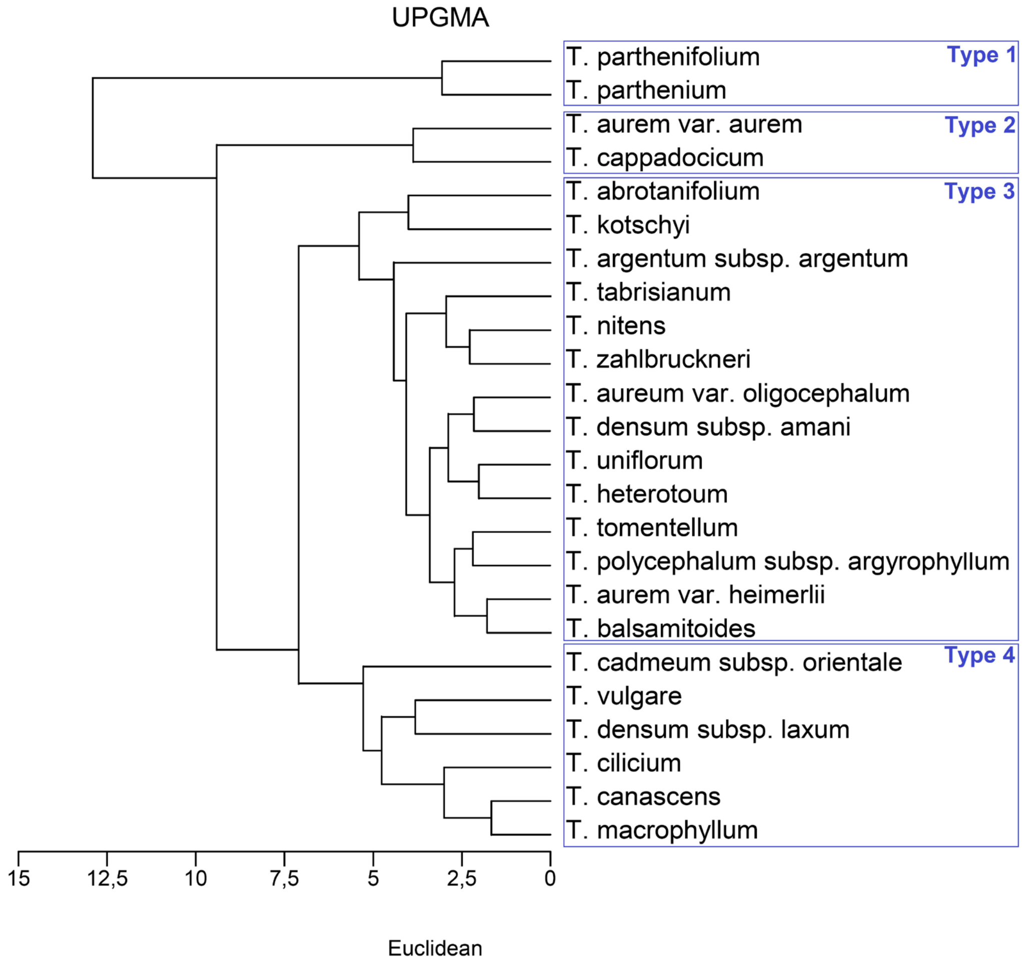

Figure 6. Dendrogram obtained from the analysis of the pollen morphological character data in the studied taxa and their related taxonomic relationships. Type 1 included subtypes *T. parthenifolium* and *T. parthenium*; type 2 included *T. aurem* var. *aurem* and *T. cappadocicum*; type 3 included *T. abrotanifolium*, *T. kotschyi*, *T. argentum* subsp. *argentum*, *T. polycephalum* subsp. *argyrophyllum*, *T. tabrisianum*, *T. aureum* var. *oligocephalum*, *T. densum* subsp. *amani*, *T. uniflorum*, *T. heterotoum*, *T. nitens*, *T. zahlbruckneri*, *T. aurem* var. *heimerlii*, *T. balsamitoides* and *T. tomentellum*; type 4 included other subtypes.

number at the base of the spines, seriate perforations in the base of the spines, and ornamentations between spines can be used as interspecific or intraspecific classification for genus *Tanacetum* taxa.

The pollen size, exine thickness, and intine thickness, as well as the form of polar and equatorial appearances, were found to be homogenous in our investigation, as in previous studies by Özmen et al. (2009) and Kodak et al. (2012). In addition, the polar axis, equatorial diameters, and intine thickness of the examined *Tanacetum* taxa were found to be smaller than those of other genera in the Asteraceae family (Shabestari et al., 2013; Bıyıklıoğlu et al., 2018; Sánchez-Chávez et al., 2022). Nonetheless, Meo (2009) discovered that the exine thickness (5.0–7.0 µm), polar axis (28.3 µm), and equatorial diameter (25.9 µm) of *Pyrethrum tatsiense* Bur et French. were longer than those investigated *Tanacetum* taxa in our study. Similarly, Meo and Khan (2006) found that *Chrysanthemum* species have longer exine thicknesses (7.9 and 9.5 µm) than our results. Furthermore, Al-Fredan (2019) discovered that the polar axis of the Asteraceae species ranged from 18.5 µm to 23.4 µm and the equatorial diameter from 19.8 µm to 26.7 µm. On the other hand, the length of the colpus, as well as the length and width of the pores in the *Tanacetum* taxa studied in this study, were found to be smaller than those of other Asteraceae family members (Montes and Murray, 2015; Bıyıklıoğlu et al., 2018). The quantity and size of colpus in Asteraceae have been documented to vary greatly (Wodehouse, 1930; Skvarla et al., 1977).

Table 4. Pollen morphological characters analyzed by principal component analysis (PCA).

PCA			**PC 1**	**PC 2**
Eigenvalues			27.625	2.234
Percentage			82.374	6.662
Cumulative Percentage			82.374	89.036
PCA variable loadings				
Morphological characters		**Characters**	**PC 1**	**PC 2**
Polar axis		P	0.458	–0.143
Equatorial diameter		E	0.525	–0.038
Porus length		Plg	0.222	0.408
Porus width		Plt	0.154	0.318
Colpus length		Clg	0.343	0.129
Colpus width		Clt	0.156	0.330
Exine thickness		Ex	0.012	–0.015
Intine thickness		In	0.009	–0.008
Apocolpium		t	0.130	0.026
Mesocolpium		L	0.505	–0.300
Operculum diameter		Op	0.139	0.348
Spine length		Sh	0.020	–0.188
Spine width		St	0.018	–0.313
Distance between spines		Ds	0.083	–0.490
PCA case scores				
Type	**Studied taxa**	**Number of taxa**	**PC 1**	**PC 2**
3	*T. abrotanifolium*	22	1.011	0.419
3	*T. argentum* subsp. *argentum*	23	0.415	0.155
2	*T. aurem* var. *aurem*	14	2.1	0.057
3	*T. aurem* var. *heimerlii*	16	0.465	0.109
3	*T. aureum* var. *oligocephalum*	15	0.105	0.006
3	*T. balsamitoides*	3	0.673	0.166
4	*T. cadmeum* subsp. *orientale*	11	–1.054	0.133
4	*T. canascens*	19	–1.043	–0.454
2	*T. cappadocicum*	9	1.565	–0.437
4	*T. cilicium*	2	–0.805	0.087
3	*T. densum* subsp. *amani*	12	–0.034	0.211
4	*T. densum* subsp. *laxum*	13	–0.42	0.651
3	*T. heterotoum*	8	–0.149	–0.243
3	*T. kotschyi*	7	1.056	0.518
4	*T. macrophyllum*	1	–1.052	–0.404
3	*T. nitens*	10	0.9	–0.452
1	*T. parthenifolium*	5	–2.658	0.037
1	*T. parthenium*	4	–2.128	0.101
3	*T. polycephalum* subsp. *argyrophyllum*	21	0.242	0.003
3	*T. tabrisianum*	20	0.376	–0.413
3	*T. tomentellum*	24	0.23	0.259
3	*T. uniflorum*	17	–0.03	–0.251
4	*T. vulgare*	18	–0.569	0.044
3	*T. zahlbruckneri*	6	0.803	–0.304

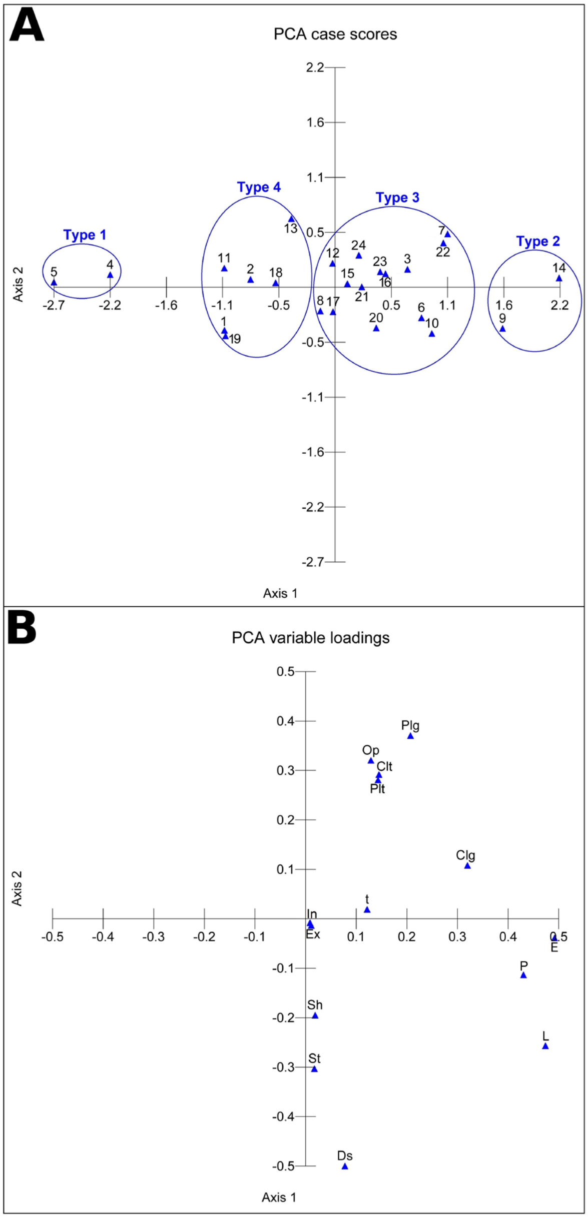

Figure 7. Principal component analysis scatter plot obtained from the overlapping of taxa. **1**: *T. macrophyllum*, **2:** *T. cilicium*, **3**: *T. balsamitoides*, **4**: *T. parthenium*, **5**: *T. parthenifolium*, **6:** *T. zahlbruckneri*, **7**: *T. kotschyi*, **8**: *T. heterotoum*, **9**: *T. cappadocicum*, **10**: *T. nitens*, **11**: *T. cadmeum* subsp. *orientale*, **12**: *T. densum* subsp. *amani*, **13**: *T. densum* subsp. *laxum*, **14**: *T. aurem* var. *aurem*, **15**: *T. aureum* var. *oligocephalum*, **16**: *T. aurem* var. *heimerlii*, **17**: *T. uniflorum*, **18**: *T. vulgare*, **19**: *T. canascens*, 20: *T. tabrisianum*, 21: *T. polycephalum* subsp. *argyrophyllum*, 22: *T. abrotanifolium*, **23**: *T. argentum* subsp. *argentum*, **24**: *T. tomentellum*, P: polar axis, E: equatorial diameter, Plg: porus length, Plt: porus width, Clg: colpus length, Clt: colpus width, Ex: exine thickness, In: intine thickness, t: apocolpium, L: mesocolpium, Op: operculum diameter, Sh: spine length, St: spine width and Ds: distance between spines.

Based on the results of a UPGMA of the pollen morphology in our study showed that morphologically similar species clustered in separate groups. However, a molecular study by Sonboli et al. (2011) found that *T. parthenifolium* and *T. parthenium* grouped, while *T. polycephalum* subsp. *argyrophyllum* and *T. uniflorum* clustered together, similar to our UPGMA results. Olanj et al. (2015) revealed that *T. parthenifolium* and *T. parthenium* have a smaller genome size than the other species evaluated in their study, and they indicated that pollen size is correlated with the genome size in *Tanacetum*. Also, Olanj et al. (2015) demonstrated that pericentromeric GC-rich heterochromatin was detected in closely related taxa such as *T. polycephalum* and *T. aureum*.

Similarly, our study showed that *T. polycephalum* subsp. *argyrophyllum* and *T. aureum* var. *heimerlii* are closely clustered. Furthermore, results from PCA were identical to those from UPGMA, and the PCA analysis suggests that pollen sizes help aggregate these taxa.

5. Conclusion

The present study proved that the spine characteristics of the *Tanacetum* taxa studied differ and can be used in classification, as well as that the spines of the investigated taxa are larger than those of various other Asteraceae taxa. The ornamentation of studied *Tanacetum* taxa was found to be echinate. In addition, with a few exceptions (suboblate), the pollen morphology of the *Tanacetum* taxa analyzed in our study was oblate-spheroidal. In the *Tanacetum* taxa examined in this study, the colpus and the porus length and width were discovered to be smaller than those of other Asteraceae family members. Similarly, the investigated *Tanacetum* taxa had a smaller polar axis, equatorial diameters, and intine thicknesses than other genera in the Asteraceae family. These features can be used in the systematics of genera and families.

Moreover, taxa are classified into different types based on PCA and UPGMA results, and pollen size plays a role in creating these types. At the species, subspecies, and genus level, the pollen characteristics produced advantageous findings. As a result, research on pollen from species of the genus *Tanacetum* can continue in the future. These studies can also be supported by molecular and seed surface research, etc. and help to systematically evaluate the genus.

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