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Leaf Micromorphology of Some *Tanacetum* L. (Asteraceae) Taxa in Turkey

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Abstract

In this study we have examined leaf micromorphology of eight taxa of *Tanacetum* L. which are distributed in Turkey. The taxa are *T. balsamita* L. subsp. *balsamita*, *T. balsamita* L. subsp. *balsamitoides*, *T. argenteum* subsp. *flabellifolium*, *T. argenteum* subsp. *argenteum*, *T. argenteum* subsp. *canum*, *T. depauperatum*, *T. haradjanii*, *T. tomentellum*. Although the leaf characteristic has rather limited systematic value, the combination of some of these features could be systematically relevant, especially for the identification of species. According to our findings the leaves of eight taxa are amphistomatic and covering trichomes very frequent and unicellar. Our goal is contribute to taxonomy of *Tanatcetum* L. which has systematic problems.

1. INTRODUCTION

Asteraceae (Compositae) family consists of approximately 1535 genus and 25000-30000 species around the World [1]. It is represented by 138 genus and 1186 species in Turkey [2,3]. The genus *Tanacetum* L. contains about 160 species and is the third largest genus of Compositae-Anthemideae, following the species rich genera *Artemisia* L. (522 species) and *Anthemis* L. (175 species) [4]. Member of this genus are distrubuted in the circum-Mediterranean region, central and eastern Asia and parts of nothern America [5]. Although most of the species are perennial herbs, the genus includes also two annuals and a number of subshrubs.

Because of its high morphological diversity and complex taxonomical history, *Tanacetum* should be considered the most problematic in this medium-sized tribe with its 111 genera and over 1700 species. The genus *Tanacetum* is in the tribe Anthemideae which contains about 10% of the total genera and 15% of the species of Asteraceae [6,7]. About 150 species of *Tanacetum* are spreaded around the World [8].

Some of the researchers place the genus different subtribes in Anthemideae. Bremer and Humphries (1993) place in the genus to Tanacetinae subtribe [9]. Then Oberprieler and his colleagues put the genus into Anthemidinae subtribe [5]. According to Turkey Vascular Plant List, the genus has 46 species, 18 subspecies and 5 variety. 26 taxon are endemic and ratio of endemism is 45 % [10].

Members of *Tanacetum* genus (Asteraceae) is a medicinal plant traditionally used for the treatment of fevers, migraine headaches, rheumatoid arthritis, stomach aches, toothaches, insect bites, infertility, and problems with menstruation and labor during childbirth. *Tanacetum* herb has a long history of use

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in traditional and folk medicine, especially among Greek and early European herbalists. *Tanacetum* has also been used for psoriasis, allergies, asthma, tinnitus, dizziness, nausea, and vomiting [11].

The objective of this experimental work is therefore to present a detailed description of leaf micromorphology for *Tanacetum* by using both scanning electron microscopy (SEM) and light microscopy (LM). With this study, we hope that make a contribution to *Tanacetum*'s problematic taxonomy.

2. MATERIAL AND METHODS

2.1 Plant Material

This study was conducted mainly on material taken from herbarium specimens. Collectors and localities of the investigated specimens are given in Tab. 1.

Table 1. Locality and voucher of *Tanacetum* taxa.

Taxa Name	Locality	Voucher
Tanacetum balsamita L. subsp. *balsamita* L	C5 Konya Karaman 950m	M. Koyuncu AEF 12691
T. balsamita L. subsp. *balsamitoides* (Schultz Bip.) Grierson	C9 Hakkari	M. Koyuncu AEF 20055
	B8 Erzurum	M. Koyuncu AEF 14165
	B6 Sivas, Kangal	N. Celik AEF 20056
	A7 Gumushane	N. Celik AEF 20057
T. argenteum (Lam.) Willd. subsp. *argenteum* (L.) All	C6 Kahramanmaras, Goksun	N. Adiguzel GAZI
	B6 Kayseri	N. Celik AEF 20285
	C9 Hakkari	M. Koyuncu AEF 26003
T. argenteum (Lam.) Willd. subsp. *flabellifolium* (Boiss. et Heldr.) Grierson	C4 Konya, Ermenek	N. Adiguzel GAZI
	C4 Konya, Ermenek	M. Coskun AEF 12647
T. argenteum (Lam.) Willd. subsp. *canum* (C. Koch) Grierson	C5 Nigde	N. Adiguzel GAZI
	C5 Icel	N. Celik AEF 26005
	B7 Erzincan, Kemaliye	O.Soner AEF 6613
	C3 Antalya, Kemer	N. Çelik AEF 26004
T. depauperatum (Post) Grierson	C6 Hatay	Baris Bani
T. haradjanii (Rech. Fil) Grierson	C6 Adana	N. Celik AEF 20048
	B6 Adana	N. Celik AEF 20049
	B6 Adana, Saimbeyli	N. Adiguzel GAZI
T. tomentellum (Boiss.) Grierson	B9 Bitlis	H.Pesmen HUB 3237

In this study, 8 taxa belonging to the genus *Tanacetum* were examined; *T. balsamita* L. subsp. *balsamita* L., *T. balsamita* L. subsp. *balsamitoides* (Schultz Bip.) Grierson, *T. argenteum* (Lam.) Willd. subsp. *flabellifolium* (Boiss. End Heldr.) Grierson, *T. argenteum* (Lam.) Willd. subsp. *argenteum* (L.) All., *T. argenteum* (Lam.) Willd. subsp. *canum* (C. Koch) Grierson, *T. depauperatum* (Post) Grierson, *T. haradjanii* (Rech. Fil.) Grierson and *T. tomentellum* (Boiss.) Grierson. *T.*

argenteum subsp. *flabellifolium, T. argenteum* subsp. *argenteum, T. depauperatum, T. haradjanii* are local endemic species. *T. balsamita* L. subsp. *balsamita* L., *T. balsamita* L. subsp. *balsamitoides* (Schultz Bip.) Grierson, *T. argenteum* (Lam.) Willd. subsp. *canum* (C. Koch) Grierson and *T. tomentellum* (Boiss.) Grierson are widely distributed, nonendemic taxa (Fig. 1).

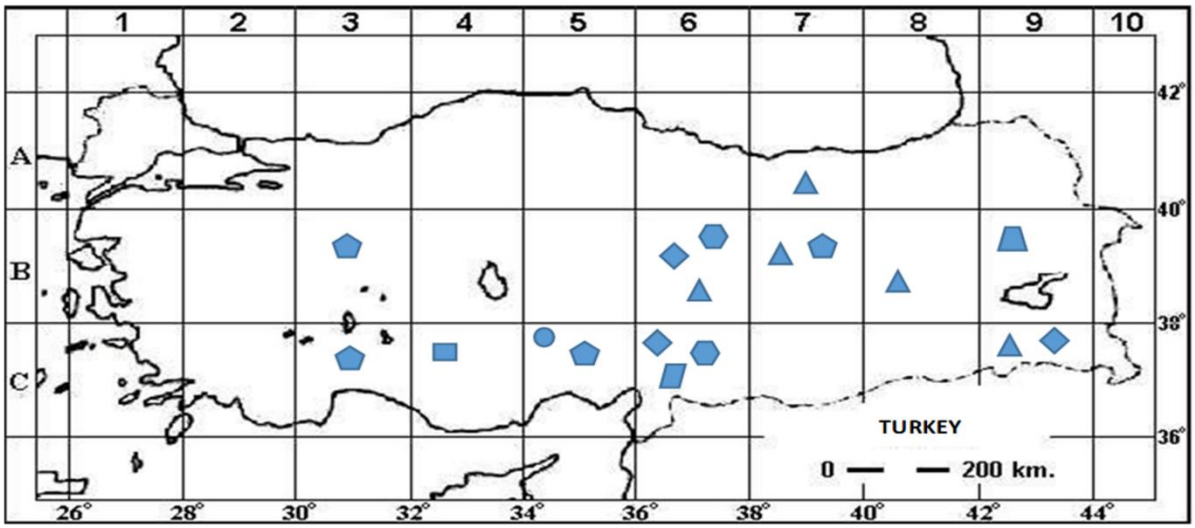

Fig 1. Turkish Grid System. *Tanacetum balsamita* L. subsp. *balsamita* L , *T. balsamita* L. subsp. *balsamitoides* , *T. argenteum* (Lam.) Willd. subsp. *argenteum* (L.) , *T. argenteum* (Lam.) Willd. subsp. *flabellifolium* , *T. argenteum* (Lam.) Willd. subsp. *canum* , *T. depauperatum* ,*T. haradjanii* , *T. tomentellum* .

2.2 Screening Methods

In this study, light microscopy (LM) and scanning electron microscopy (SEM) have been used for detailed examining leaf micromorphology of *Tanacetum*.

2.3 LM Analysis

Leaf micromorphological studies were carried out on herbarium specimens. The superficial sections of leaves were done by hand and slides were photographed using Leica S8 APO apochromatic stereo microscope and DM 1000 light microscope with Leica DSCF320 camera.

2.4 SEM (Scanning Electron Microscopy) Method

The scanning electron microscopy studies was performed in TPAO (Turkish Petroleum Incorporated Company) Laboratory. The leaf samples were mounted on double-stick and pulse sputter-coated for 3,5 minutes with gold. Leaf samples were analyzed with JSM-6060 Jeol SEM. Detailed leaf surface ornamentation has been examined with this technique. We have benefited from following articles to interpret leaf micromorphology results, [12], [13], [14], [15], [16].

2.5 Numerical analysis

For the leaf micromorphological characters of the 8 taxa coefficients of correlation were determined, and they were grouped using the clustering analysis method (UPGMA, dissimilarity, standardized variables). A total of 5 leaf micromorphological characters comprising 3 quantitative and 2 qualitative characters were selected to distinguish 8 studied taxa of *Tanacetum* (Tab. 2). For the multivariate

analysis, a primary matrix was created using 8 taxa and five characters. The clustering analysis was based on Gower's (1971) [17] general coefficient similarity [18], which can be used directly with a mixture of character types (binary, qualitative and quantitative characters). UPGMA was selected because it is not only the most commonly used method but also appears to produce an accurate reflection similarity matrix as measured by the co-phenetic correlation coefficient of Sokal and Rohlf (1962) symmetrical hierarchical structure and congruence with classification derived by traditional methods [19-21]. All computations were made by the MVSP 3.1 software.

Table 2. *Summary statistics for five characters examined in 8 taxa of Tanacetum*

Taxa/Variables	Hair frequency	Hair count	Stomata length	Stomata width	Stomata count
T. balsamita* subsp. *balsamita	0.000	9.000	33.000	23.000	0.000
T. balsamita* subsp. *balsamitoides	1.000	5.000	24.000	21.500	0.000
T. argenteum* subsp. *flabellifolium	0.000	9.000	21.000	16.500	1.000
T. argenteum* subsp. *argenteum	0.000	10.000	24.500	14.000	2.000
T. argenteum* subsp. *canum	0.000	9.000	19.000	11.000	1.000
T. depauperatum	0.000	11.000	22.500	18.500	0.000
T. haradjanii	0.000	13.000	16.500	11.000	2.000
T. tomentellum	0.000	11.000	17.000	19.000	3.000

3. RESULTS AND DISCUSSION

Light and scanning electron microscopy were used to analyze the micromorphological characters of the leaves of each *Tanacetum* taxa. The leaf characters studied showed little variations. The cuticular membrane has ornamentation in all the examined taxa. Epidermis is single cell layer thick and cell sizes are various. The average number of cells in the upper and lower surfaces of epidermis were found 4-6 per μm^2. The taxa examined have amphistomatic leaves. Stomata cells are at the same level with epidermis cells. Stomata types for all the examined taxa is anamocytic. The length of stomata is range from 15 to 35 μm, the width of stomata is 10-25 μm. Stomata numbers per mm^2 are range from 3 to 5. Covering trichomes are very frequent (8-12 mm^2) and unicellular. The length of trichomes varies from 500 μm to 800 μm. *T. balsamita* subsp. *balsamitoides* has rare (4-6 mm^2) and shorter (250 to 500 μm) glandular hairs. In *T. balsamita* subsp. *balsamita*, *T. balsamita* subsp. *balsamitoides* and *T. deparatum* trichome walls are uncertain striate. In *T. argentea* subsp. *flabellifolium*, *T. argentea* subsp. *argenteum*, *T. argentea* subsp. *canum*, *T. haradjani* and *T. tomentellum*, trichome walls are striate (Fig 2, 3, 4). Karamian et al. (2009) stated that the ornamentations are diagnostic feature for the leaf trichomes [22]. *T. argentea* subsp. *argenteum*, T. argentea subsp. *canum* and *T. haradjanii* have globular glandular hairs (Tab. 3).

The result of the study show that dendrogram, generated from leaf micromorphological characters constructed by UPGMA, revealed three main groups with 60 % level of similarity; the first one comprises *T. balsamita* subsp. *balsamitoides* which is seperated from the others *at first*, the second one comprises *T. balsamita* subsp. *balsamita* and the third one comprises other 6 taxa. The highest similarity is about 90% between *T. argentea* subsp. *flabellifolium* and *T. argentea* subsp. *canum*. *T. argentea* subsp. *argenteum* is closely related with this both with 80% similarity. Also *T. depauperatum* is qui-te similar to this group with a degree about 75%. *T. tomentellum* and *T.*

haradjani are quite similar with a degree of 75 % similarity (Fig. 5). Because there is limited research about the leaf micromorphology or anatomy of *Tanacetum*, our study has great importance in order to contribute to taxonomy of the genus.

Adedeji and Jewoola (2008), have examined stomata properties of 13 taxon of Asteraceae, and they found that those 13 taxon have amphistomatic leaf and anomocytic type of stomota [23]. Brown (1999) and Majdii (2010) have observed secretory hair on *Tanacetum parthenium* and *Tanacetum vulgare* species [24,25]. Kadiri et al. (2005) have described the leaf micromorphologies of Nigeria *Synedrella* (Linn.) Gaertn. taxa in LM [26]. Adedeji and Jewoola (2008) analysed the epidermal structure of leaves in Asteraceae family [23]. Noorbakhsh et al. (2008) examined leaf micromorphologies of 28 Iranian *Artemisia* species by light microscopy[27]. Makbul et al. (2011) have analysed that leaf micromorphology of 18 taxon of *Scorzonera* L. which are distrubuted in Turkey[28]. Inceer and Ozcan have performed a study on leaf anatomy of 18 taxon of *Matricaria* L. and *Tripleurospermum* Schultz Bip [29]. Majdii et al. (2010) have studied induction of tetraploidy to feverfew (*Tanacetum parthenium* Schulz-Bip.): morphological, physiological, cytological, and phytochemical changes [25]. Stevovic et al. (2010) have examined that how environmental effects affect on leaf anatomy of *Tanacetum vulgare* which is growth in two different localities [30]. Kodak et. al. (2012) have investigated pollen morphologies eight taxa of *Tanacetum* genus and they found that the pollen was tricolporate, trisyncolporate or tricolpate. The shape was oblate- spheroidal. The exine was echinate. The ornamentations between spines were granulate, reticulate and rugulate-granulate [8]. In this study we have examined leaf micromorphology of eight taxa of *Tanacetum* which are spread out in Turkey. The taxa are *T. balsamita* L. subsp. *balsamita*, *T. balsamita* L. subsp. *balsamitoides* (Schultz Bip. Grierson), *T. argenteum* (Lam. Willd.) subsp. *flabellifolium* (Boiss. ve Heldr. Grierson), *T. argenteum* (Lam. Willd.) subsp. *argenteum*, *T. argenteum* (Lam. Willd.) subsp. *canum* (C. Koch Grierson), *T. depauperatum* (Post Grierson), *T. haradjanii* (Rech. fil. Grierson), *T. tomentellum* (Boiss Grierson).

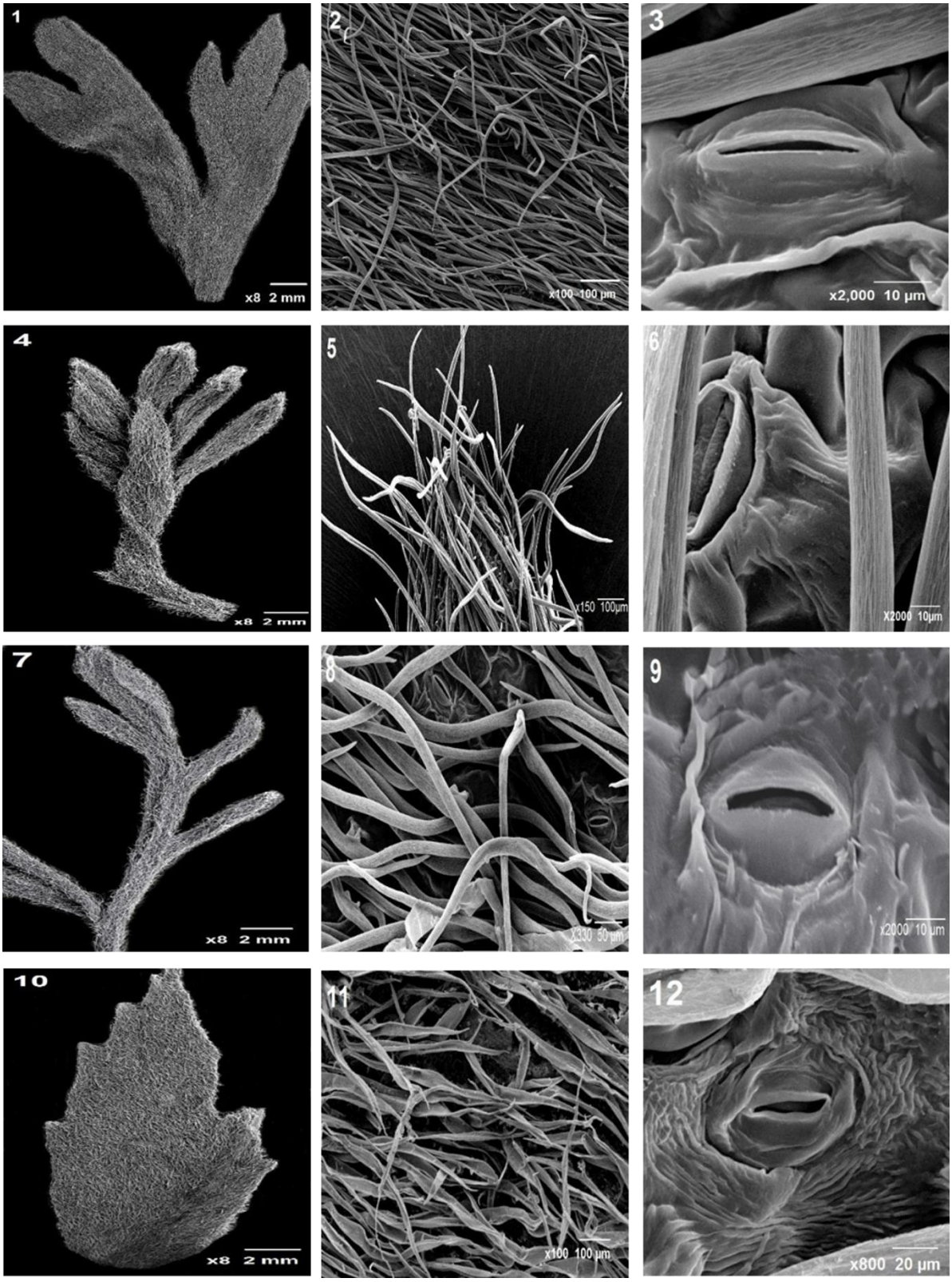

Fig 2. *Leaf Micromorphologies of Tanacetum by electron microscopy (SEM).* ***1-3*** *T. argenteum subsp. argenteum;* ***4-6*** *T. argenteum subsp. canum;* ***7-9*** *T. argenteum subsp. flabellifolium;* ***10-12*** *T. balsamita subsp. balsamita*

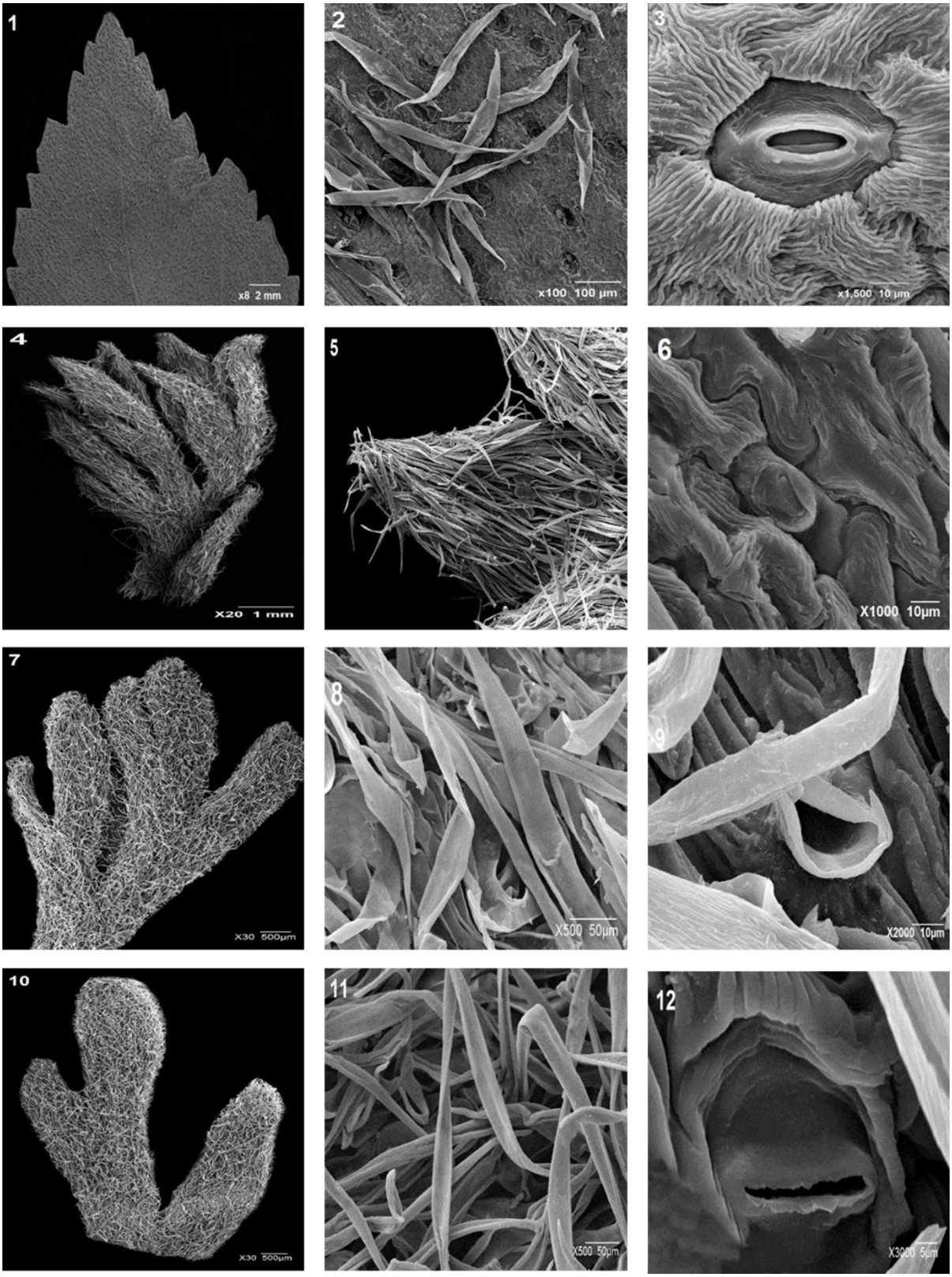

Fig 3. *Leaf Micromorphologies of Tanacetum by Scanning Electron Microscope (SEM).* ***1-3*** *T. balsamita L. subsp. balsamitoides;* ***4-6*** *T. depauperatum;* ***7-9*** *T. haradjanii;* ***10-12*** *T. tomentellum*

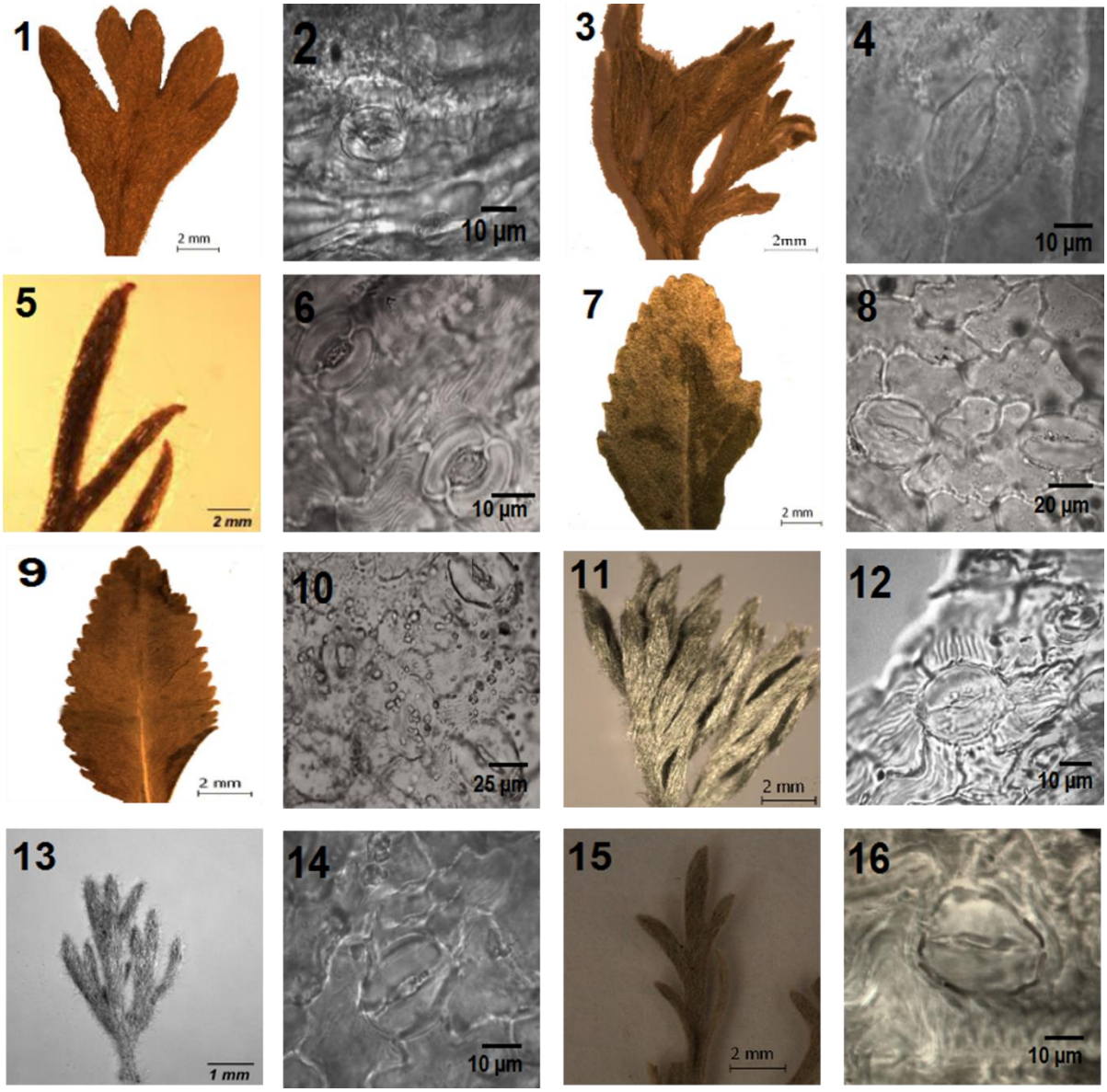

Fig 4. *Leaf Micromorphologies of Tanacetum by Light Microscope (LM).* ***1-2*** *T. argenteum subsp. argenteum;* ***3-4*** *T. argenteum subsp. canum;* ***5-6*** *T. argenteum subsp. flabellifolium;* ***7-8*** *T. balsamita subsp. balsamita;* ***9-10*** *T. balsamita L. subsp. balsamitoides;* ***11-12*** *T. depauperatum;* ***13-14*** *T. haradjanii;* **15-16** *T. tomentellum*

Table 3. *Leaf micromorphological characteristics of Tanacetum taxa examined*

	Epidermal cell	Hair				Stomata					
Taxo Names	Count (mm²)	Frequency	Length (µm)	Count (mm²)	Ornamentation	Length (µm)	Width (µm)	Count (mm²)	Level According to Epidermis	Type of Stomata	Type of Leaves
T. balsamita subsp. *balsamita*	4-6	Frequent	500-800	8-10	Striate	30-35	21-25	3-4	Same level	Anomocytic	Amphistomatic
T. balsamita subsp. *balsamitoides*	4-6	Rare	250-500	4-6	Striate	23-25	21-22	3-4	Same level	Anomocytic	Amphistomatic
T. argenteum subsp. *flabellifolium*	4-6	Frequent	500-800	8-10	Striate	20-22	15-18	4-5	Same level	Anomocytic	Amphistomatic
T. argenteum subsp. *argenteum*	4-6	Frequent	500-800	9-11	Striate	23-26	13-15	4-6	Same level	Anomocytic	Amphistomatic
T. argenteum subsp. *canum*	4-6	Frequent	500-800	8-10	Striate	18-20	10-12	4-5	Same level	Anomocytic	Amphistomatic
T. depauperatum	4-6	Frequent	500-800	10-12	Striate	20-25	17-20	3-4	Same level	Anomocytic	Amphistomatic
T. haradjanii	4-6	Frequent	500-800	12-14	Striate	15-18	10-12	2-3	Same level	Anomocytic	Amphistomatic
T. tomentellum	4-6	Frequent	500-800	10-12	Striate	16-18	18-20	3-5	Same level	Anomocytic	Amphistomatic

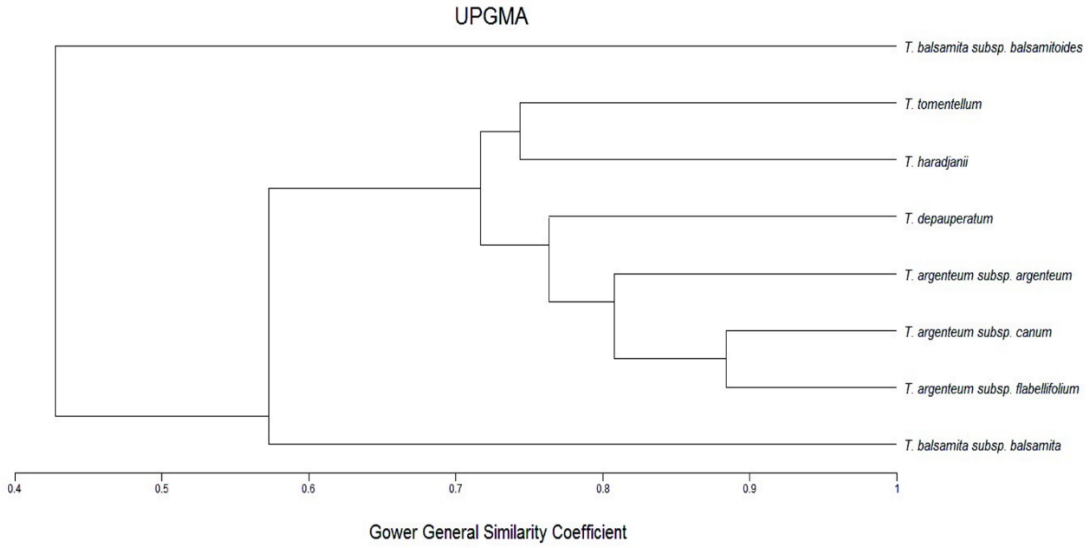

Fig 5. *Dendrogram showing similarity distance of the examined taxa of Tanacetum.*

Although the leaf characteristic has rather limited systematic value, the combination of some of these features could be systematically relevant, especially for the identification of species. According to our findings the leaves of eight taxa are amphistomatic and covering trichomes very frequent and unicellar. Our goal is contribute to taxonomy of *Tanacetum* which has systematic problems.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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