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Leaf anatomy as an additional taxonomy tool for 18 taxa of *Matricaria* L. and *Tripleurospermum* Sch. Bip. (Anthemideae-Asteraceae) in Turkey

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Abstract This study concerns the evaluation of leaf anatomical characteristics of the closely related genera *Matricaria* (4 taxa) and *Tripleurospermum* (14 endemic taxa, 15 accessions) in Turkey using cluster analysis (CA) and principal component analysis (PCA). All taxa have the same basic leaf structure composed of lower and upper epidermis including anomocytic or anomocytic-anisocytic stomata, parenchymatic mesophyll, and vascular bundle. Statistically significant differences based on ploidy level have been found without generic delimitation. The differences mainly concern stomatal length and the relative size of the vascular bundle. These anatomical characters can be used to distinguish diploid from polyploid taxa of the genera.

Keywords Anatomy · Leaf · *Matricaria* · Ploidy level · Taxonomy · *Tripleurospermum* · Turkey

Introduction

The tribe Anthemideae Cass. of the family Asteraceae comprises 111 genera and ca. 1,800 species that are distributed worldwide (extratropical) but mainly in central Asia, the Mediterranean, and southern Africa (Oberprieler et al. 2007; Himmelreich et al. 2008). It is generally accepted as a relatively natural assemblage.

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The genera *Matricaria* L. and *Tripleurospermum* Sch. Bip. belong to the tribe Anthemideae and comprise ca. six and 40 species, respectively, which are distributed mainly in Europe, temperate Asia, North America, and North Africa (Oberprieler et al. 2007). It is difficult to determine the exact number of species without a monographical treatment of the whole genera because their species have often been referred to other Anthemideae genera such as *Anthemis* L., *Pyrethrum* Zinn., *Chrysanthemum* L., and *Chamaemelum* Mill. (Pobedimova 1995; Inceer and Hayirlioglu-Ayaz 2010).

Taxonomic problems of these genera cannot be ignored. Species of *Matricaria* and *Tripleurospermum* are similar to each other in their morphological characteristics such as floral architecture and leaf shape and also resemble many other Anthemideae genera in their habit. Therefore, they have been confused both taxonomically and nomenclaturally with each other and other Anthemideae genera (Jeffrey 1979; Xifreda 1985; Kerguelen et al. 1987; Pobedimova 1995; Applequist 2002).

The taxonomy of these genera continues to be the subject of much confusion, mainly because of the approaches to species delimitation that have been employed, resulting in different numbers of recognized species. Initially, *Tripleurospermum* was assigned to the genus *Matricaria*, but later was recognized as a separate genus on the basis of the different structure of its achenes and the occurrence of a tetrasporic embryo sac (Harling 1951). However, Rauschert (1974) and Kay (1976) misapplied the name *Matricaria* to refer exclusively to the species of *Tripleurospermum* (Bremer and Humphries 1993). Bremer and Humphries (1993) and Applequist (2002) considered that the two genera were closely related to each other, and thus they were classified in the same subtribe Matricariinae. In contrast,

Oberprieler (2001), Oberprieler and Vogt (2006), Oberprieler et al. (2007), and Lo Presti et al. (2010) showed that *Tripleurospermum* is closely related to *Anthemis* in the strict sense and not to *Matricaria* based on molecular markers. This is supported by the tetrasporic embryo sac shared by *Tripleurospermum* and *Anthemis* (vs. monosporic in *Matricaria* and other Anthemideae genera).

Cytological studies conducted in *Matricaria* and *Tripleurospermum* have concentrated on the chromosome count, with little work having been focussed on detailed karyological criteria for taxonomic purposes (Watanabe 2009 and references therein). All members of the genera show a base chromosome number of $x = 9$, the most common basic number in the tribe Anthemideae and the family Asteraceae (Inceer and Hayirlioglu-Ayaz 2010). To date, one ploidy level ($2x$) in *Matricaria* and four ploidy levels ($2x$, $3x$, $4x$, $5x$) in *Tripleurospermum* have been reported from Turkey, which is regarded as one of the main centers of diversification and speciation (Inceer and Beyazoglu 2004; Garcia et al. 2005; Inceer and Hayirlioglu-Ayaz 2010). The diploid and tetraploid levels are more common than the triploid and pentaploid levels in *Tripleurospermum*. The stability of ploidy level in *Matricaria* is noteworthy in Turkish populations (Inceer and Hayirlioglu-Ayaz 2010).

Stomatal frequency, guard cell length, and stomatal plastid number have been used as morphological markers for identifying ploidy levels in many plant species (Bingham 1968; Santen and Casler 1986; Mishra 1997; Beck et al. 2003). It was noted that the leaves of polyploids were usually thicker and firmer than those of their diploid progenitors (Stebbins 1971). A positive relationship was also found between ploidy level and stomatal length in *Tripleurospermum* (Inceer and Hayirlioglu-Ayaz 2010).

Although the leaf is the organ that anatomically differs the most based on the hierarchic level, i.e., species, genus, and family, and despite the influence of environmental factors, many anatomical characters are useful for systematics, particularly the leaf epidermis (Metcalf and Chalk 1979; Dickison 2000; Araújo et al. 2010). In terms of leaf anatomy as it applies to taxonomy, few references are found for *Matricaria* and *Tripleurospermum* (Napp-Zinn and Eble 1978; Uysal 1991; Inceer and Hayirlioglu-Ayaz 2010).

The objectives of present work are to (1) study the leaf structure in 18 taxa of *Matricaria* and *Tripleurospermum* with known or unknown chromosome numbers from Turkish populations in order to find new information and determine the variation among the taxa based on ploidy level, and (2) discuss the inter- and intrageneric relationships based on anatomical data.

Materials and methods

Plant materials

The materials were collected from natural populations in Turkey, all of which are cytologically known except *M. chamomilla* var. *chamomilla* and *T. fissurale*. Vouchers are deposited in the herbarium at Karadeniz Technical University, Department of Biology (KTUB) or H. Inceer collections (Table 1).

Anatomical studies

For the anatomical analysis, 14 fresh leaves of four different specimens per population were fixed in FAA (5 parts formalin: 5 parts acetic acid: 90 parts 70% ethyl alcohol) immediately after detachment. After being fixed for at least 48 h, plant materials were transferred to 50% ethyl alcohol, dehydrated with ascending alcohol series, and embedded in paraffin (Lu et al. 2008). Transverse sections, 18–22 μm thick, were made with a rotary microtome and stained in hematoxylin (Algan 1981). The dimensional characteristics of the transverse sections were measured using a Nikon binocular microscope with an ocular micrometer, and original drawings were made by means of a light microscope provided with a camera lucida.

Paradermal sections were stained in hematoxylin for about 10 min, and temporary slides were mounted in glycerine. Ten slides were prepared for each taxa, and five stomatal lengths were measured on each slide in random fields of view. Stomatal measurements were carried out on the upper (adaxial) epidermis of many of the fresh or dried leaves. Stomatal index was assessed in both the upper and lower (abaxial) epidermis (Meidner and Mansfield 1968). Paradermal sections were observed under 40 \times magnification using Olympus BH-2 light microscope with an ocular scale. Drawings were made using a camera lucida.

Chromosome counts

Root tip meristems obtained from germinated achenes were used for chromosome analysis. Samples were pre-treated with 0.05% colchicine for 2–5 h. They were fixed in absolute ethanol-glacial acetic acid (3:1) for at least 24 h at 4°C, hydrolysed in 1 N HCl at 60°C for 10 min and then rinsed in tap water for 2–3 min (Inceer and Hayirlioglu-Ayaz 2007). Staining was carried out in 1% aqueous aceto-orcein for 12–18 h at room temperature, squashes were made in 45% acetic acid, and the preparations were mounted in Entellan. Permanent slides were prepared from at least ten well-spread metaphase plates.

Table 1 The collections data of the investigated *Matricaria* and *Tripleurospermum*

Taxon	Locality	Voucher
<i>Matricaria aurea</i> (L.) Sch. Bip.	Gaziantep/Şanlıurfa: between Nizip and Birecik, Dutlu, roadsides, near cultivated area, 440 m, 08.v.2007, N37°00'50.4", E37°52'01.4"	Inceer 322
<i>M. chamomilla</i> L. var. <i>chamomilla</i>	Çanakkale: Koru Dağı, near <i>Pinus brutia</i> forest, 70 m, 11.v.2007, N40°42'58.1", E26°44'15.0"	Inceer 331
<i>M. chamomilla</i> L. var. <i>recutita</i> (L.) Fiori	Izmir: Odemis, between Izmir and Odemis, roadsides and cultivated fields, 17 m, 14.iv.2007, N38°06'35.6", E27°25'31.1"	Inceer 258
<i>M. matricarioides</i> (Less.) Porter ex Britton	Kars (Ardahan): between Ardahan and Göle, roadsides, 1,800 m, 18.vii.2007, N41°05'45.3", E42°39'52.1"	Inceer 420
<i>Tripleurospermum baytopianum</i> E. Hossain	Tekirdağ: between Şarköy and Gölcük, slopes, meadows and roadsides, 250 m, 17.iv.2008, N40°42'58.1", E26°44'15.0"	Inceer 508
<i>T. callosum</i> (Boiss. and Heldr.) E. Hossain	Gümüşhane: Kop Dağı pass, damp alpine pastures, meadows, 2,200 m, 14.vii.2000, N40°02'13.8", E40°30' 39.5"	Inceer 069
<i>T. conoclinium</i> (Boiss. and Bal.) Hayek	Izmir: Boz Dağ, cultivated fields, meadows and pastures, 07.iv.2008, 1,178 m, N38°18'14.9", E28°03'05.4"	Inceer 478
<i>T. corymbosum</i> E. Hossain	Ağrı: Suluçem (Musun), Balık Gölü, meadows, cultivated fields, 2,098 m, 11.vii.2008, N39°41'35.8", E43°48'44.6"	Inceer 612
<i>T. fissurale</i> (Sosn.) E. Hossain	Artvin: From Yusufeli to Ispir, among stones, 542 m, 31.v.2008, N40°48'15.4", E41°39'02.9"	Inceer 535
<i>T. heterolepis</i> (Frey and Sint.) Bormm.	Gümüşhane: Keçikaya Village, roadsides, 1,618 m, 04.vii.2007, N40°22'33.6", E39°41'58.3"	Inceer 382b
<i>T. hygrophilum</i> (Bormm.) Bormm.	Izmir: Yamanlar Dağı, near <i>Pinus</i> forest, open places, 887 m, 15.iv.2007, N 38°33'36.5", E27°13'04.3"	Inceer 273
<i>T. kotschyi</i> (Boiss.) E. Hossain	Niğde: Ulukışla, Bolkar mountains, near Karagöl, 2,600 m, 29.vii.2008, N37°24'20.3", E34°33'32.3"	Inceer 702
<i>T. monticolum</i> (Boiss. and Huet) Bormm.	Trabzon: Zigana Dağı Pass, meadows, open slopes, 2,000 m, 26.vii.2002, N40°38'48.15", E39°22'35.46"	Inceer 166
<i>T. pichleri</i> (Boiss.) Bormm.	Bursa: Uludağ, meadows, damp woods, 1,828 m, 11.vi.2008, N40°06'24.5", E29°08'15.2"	Inceer 553
<i>T. repens</i> (Frey and Sint.) Bormm.	Rize: İkizdere, between Cimil and Başköy, stream sides, roadsides, 1,800 m, 11.vii.2001, N40°39'02.0", E40°32'32.0"	Inceer 133
<i>T. rosellum</i> (Boiss. and Orph.) Hayek var. <i>album</i> E. Hossain	Bolu: Near Abant Lake, meadows, 1,331 m, 12.vi.2008, N40°36'25.3", E31°16'24.4"	Inceer 555
<i>T. rosellum</i> (Boiss. and Orph.) Hayek var. <i>album</i> E. Hossain	Rize: İkizdere, between Cimil and Ortaköy, bare ground, roadsides, stony pastures on limestone, 1,750 m, 11.vii.2001, N40°45'42.8", E40°45'22.0"	Inceer 134
<i>T. ziganaense</i> Inceer and Hayirlioglu-Ayaz	Gümüşhane: Zigana Dağı, between Zigana Pass and Torul, 1,300 m, 22.vii.2008, N40°38'27.25", E39°24'15.6"	Inceer 666

Statistical analyses

Multivariate analyses were carried out to evaluate the anatomical characters of the leaf using Statistica version 7.0. For all anatomical characters (1 qualitative and 11 quantitative with mean value) and ploidy levels of the taxa, coefficient of correlation was determined, and their grouping was performed using the clustering analysis method (UPGMA, dissimilarity, standardized variable) as well as ordination based on principal component analysis (PCA).

Nomenclature

We follow herein the nomenclature adopted by Grierson (1975), Enayet Hossain (1975), and Inceer and Hayirlioglu-

Ayaz (2008). Studied taxa are listed by their genera in alphabetical order in the tables.

Results

Epidermal cells

In the studied taxa the leaf is generally slightly curved outwards with a cuticle on the other side. The cuticle is generally not very thick. However, it is thicker in *Tripleurospermum* than *Matricaria*. There is a single layered isodiametric epidermis with more or less straight walls on the adaxial and abaxial surface of the leaf in all taxa. Adaxial epidermal cells are equal to the abaxial ones, and

they are smaller and more round on the lower part of the midrib than other parts in the transverse sections. Epidermal cell walls have the same structure with anticlinal undulate to sinuate walls on both the adaxial and abaxial surface of all the studied taxa (Fig. 1). In addition, there is no difference between the adaxial and abaxial surfaces of the taxa, except for *T. fissurale* (Fig. 1). The adaxial epidermal cells of this species have slightly wavy or more or less straight walls.

Stomata

Stomata are present on both surfaces of the leaf (=amphistomatic type) in the studied taxa and lie more or less at the epidermis level (Table 2). The majority of the taxa have anomocytic stomata (stomata without subsidiary cells). However, anisocytic stomata are also observed in the adaxial surface of *T. fissurale* (Fig. 1). The guard cells in all studied taxa are oval-shaped and variable in size.

Trichomes

Leaf surfaces are more or less densely covered by uniseriate nonglandular, multicellular trichomes, forming an

indumentum of variable texture and density in the majority of the taxa. They consist of a variable number of short basal cells and an elongated, pointed terminal cell (Fig. 2). Among the 18 examined taxa, *T. corymbosum* is the only glabrous one, and the others are more or less pubescent with uniseriate trichome type. In particular, trichomes are more dense in *T. fissurale* and *T. ziganaense*.

Leaf blades

Mesophyll is generally dorsiventral, and the palisade parenchyma, which is usually mono- or bilayered, is discontinuous above the midrib. However, palisade cells are clearly prominent in *T. conoclinium*, *T. fissurale*, and *T. ziganaense* (Figs. 4, 5, 7). The midrib region is parenchymatic and triangular on the abaxial side, whereas it is flattened on the adaxial one. Its breadth ranges from 293 μm (*T. baytopianum*) to 967.5 μm (*T. rosellum* var. *album*, 4x) in *Tripleurospermum* taxa and from 336 μm (*M. aurea*) to 674 μm (*M. chamomilla* var. *recutita*) in *Matricaria* taxa (Table 2). Predominantly, mechanical tissue of a collenchymatic nature is located under the lower epidermis of the midrib region. However, the collenchyma is not found in *M. aurea* and *T. conoclinium* (Figs. 3, 4).

Fig. 1 Paradermal sections in leaves of *Matricaria* and *Tripleurospermum*. **a** *M. aurea*, **b** *M. chamomilla* var. *chamomilla*, **c** *M. chamomilla* var. *recutita*, **d** *M. matricarioides*, **e** *T. callosum*, **f** *T. fissurale* (adaxial surface), **g** *T. fissurale* (abaxial surface), **h** *T. monticolum*, **i** *T. rosellum* var. *album* (4x). Scale bars 40 μm

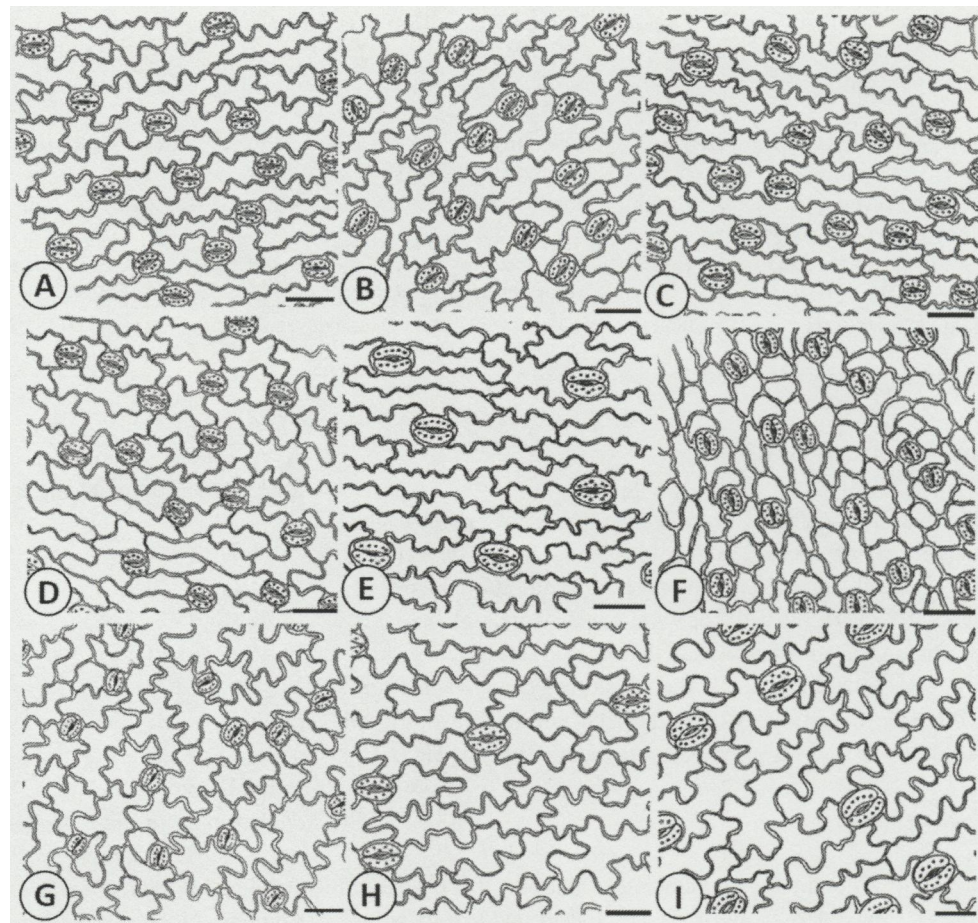


Table 2 Leaf anatomical characteristics of the investigated taxa of *Matricaria* and *Tripleurospermum*

Taxon	Cuticle thickness (μm)	Mesophyll breadth (mid) (μm)	Vascular bundles		Xylem thickness (μm)	Phloem thickness (μm)	Coll	Stomatal length (μm)	Stomata number (per 1 mm^2)		Stomatal index	
			Length (μm)	Breadth (μm)					Adaxial surface	Abaxial surface	Adaxial surface	Abaxial surface
<i>Matricaria aurea</i> (2x) ^a	1.9 \pm 0.1	336 \pm 4.60	124.1 \pm 1.90	112.5 \pm 3.60	91 \pm 1.30	32.3 \pm 1.0	-	24.7 \pm 0.7	176.6 \pm 16.0	129.7 \pm 5.90	24.2 \pm 1.1	21.3 \pm 0.6
<i>M. chamomilla</i> var. <i>chamomilla</i> (2x)	2.2 \pm 0.1	435.1 \pm 11.8	201.6 \pm 2.00	198 \pm 10.3	125.9 \pm 0.70	76 \pm 2.4	+	27.5 \pm 0.6	222.7 \pm 18.8	182.8 \pm 20.9	23.8 \pm 0.4	21.3 \pm 0.9
<i>M. chamomilla</i> var. <i>recutita</i> (2x) ^a	2.8 \pm 0.3	674 \pm 25.0	205.3 \pm 4.70	184 \pm 3.30	120.3 \pm 1.90	81.8 \pm 3.1	+	26.2 \pm 0.5	178.1 \pm 3.10	178.1 \pm 6.00	23.6 \pm 0.2	24.3 \pm 0.7
<i>M. matricarioides</i> (2x) ^a	2.6 \pm 0.2	564 \pm 9.50	180 \pm 3.70	227.6 \pm 6.90	124.8 \pm 1.50	59 \pm 2.6	+	28 \pm 0.1	204.7 \pm 5.30	142.2 \pm 13.6	21.5 \pm 0.2	15.1 \pm 0.8
<i>Tripleurospermum baytopianum</i> (2x) ^a	2.8 \pm 0.1	293 \pm 4.10	118 \pm 2.20	120.5 \pm 5.70	76.8 \pm 2.80	41.8 \pm 0.9	+	26.3 \pm 0.3	194.5 \pm 10.4	218.8 \pm 11.1	21.4 \pm 0.7	22.6 \pm 1.1
<i>T. callosum</i> (4x) ^b	3.9 \pm 0.2	491.5 \pm 13.5	293.5 \pm 2.20	291 \pm 1.90	205 \pm 2.50	85.8 \pm 2.2	+	35.4 \pm 0.5	86.7 \pm 2.00	89.8 \pm 7.10	18.1 \pm 0.5	19.3 \pm 0.6
<i>T. conolinium</i> (2x) ^a	4 \pm 0.1	626.8 \pm 8.60	150.8 \pm 1.90	204 \pm 7.50	95.5 \pm 1.00	55.3 \pm 1.1	-	29.4 \pm 0.2	153.9 \pm 12.2	109.4 \pm 6.00	21.3 \pm 0.5	15.3 \pm 0.8
<i>T. corymbosum</i> (3x) ^a	4.9 \pm 0.4	695 \pm 2.50	302.3 \pm 10.4	285 \pm 15.3	220 \pm 13.0	81.5 \pm 2.6	+	31.7 \pm 0.2	99.2 \pm 4.50	99.2 \pm 4.50	20.1 \pm 0.5	22.1 \pm 1.2
<i>T. fissurale</i> (2x)	4.6 \pm 0.4	565.4 \pm 9.70	242.8 \pm 3.80	223.2 \pm 1.10	166.5 \pm 2.00	80.5 \pm 1.5	+	25.9 \pm 0.6	165.6 \pm 18.1	152.3 \pm 10.8	23.5 \pm 1.2	19.5 \pm 1.0
<i>T. heterolepis</i> (4x) ^a	3.6 \pm 0.2	607 \pm 1.00	326.3 \pm 2.10	308 \pm 2.80	223.6 \pm 0.40	102.5 \pm 1.5	+	34.5 \pm 1.1	96.5 \pm 6.00	93 \pm 4.50	22.3 \pm 0.1	17.7 \pm 0.9
<i>T. hygrophilum</i> (2x) ^a	2.6 \pm 0.1	402 \pm 6.60	139 \pm 2.10	149.5 \pm 3.20	90.3 \pm 2.70	49.3 \pm 0.8	+	26.2 \pm 0.1	199.2 \pm 6.80	190.6 \pm 1.80	21.4 \pm 0.3	18.6 \pm 0.8
<i>T. kotschy</i> (4x) ^a	3.1 \pm 0.1	478.8 \pm 11.2	184.9 \pm 2.00	222.1 \pm 11.9	121.5 \pm 1.00	61.9 \pm 2.6	+	33 \pm 0.1	95.3 \pm 3.00	98.4 \pm 3.90	18.1 \pm 0.5	15.2 \pm 0.2
<i>T. monticulum</i> (4x) ^b	3.5 \pm 0.2	597.5 \pm 5.50	306.8 \pm 3.80	380.3 \pm 3.80	200.8 \pm 3.40	106.3 \pm 2.5	+	38.9 \pm 0.7	91.4 \pm 5.30	71.9 \pm 6.00	18.1 \pm 0.7	14.8 \pm 0.6
<i>T. pichteri</i> (4x) ^a	3.4 \pm 0.2	643 \pm 5.50	182.5 \pm 6.20	190.2 \pm 6.90	126.5 \pm 4.50	55.3 \pm 0.8	+	34 \pm 0.3	109.4 \pm 4.00	92.2 \pm 4.70	19.6 \pm 0.2	22 \pm 0.3
<i>T. repens</i> (4x) ^b	3.9 \pm 0.3	468.5 \pm 7.80	272.6 \pm 1.20	276.8 \pm 4.30	203.8 \pm 1.00	66.7 \pm 1.9	+	38.1 \pm 0.2	93.8 \pm 2.60	83.3 \pm 1.80	23.2 \pm 0.1	19 \pm 0.7
<i>T. rosellum</i> var. <i>album</i> (2x, Bolu) ^a	3.3 \pm 0.2	336 \pm 2.80	127 \pm 0.40	111.3 \pm 0.50	91.5 \pm 1.20	39 \pm 2.1	+	28.5 \pm 0.2	205.5 \pm 4.10	198.4 \pm 4.70	23.5 \pm 0.3	24.1 \pm 1.3
<i>T. rosellum</i> var. <i>album</i> (4x, Rize) ^b	4.1 \pm 0.1	967.5 \pm 26.7	431.3 \pm 3.80	562.5 \pm 9.30	297 \pm 2.50	135.8 \pm 2.0	+	39.8 \pm 0.2	75 \pm 5.10	60.9 \pm 5.30	20.5 \pm 0.4	10.4 \pm 0.5
<i>T. ziganaense</i> (2x) ^a	5.4 \pm 0.2	341.2 \pm 8.20	107.8 \pm 3.10	112.3 \pm 3.40	75.8 \pm 0.30	32 \pm 2.8	+	28.9 \pm 0.3	125.8 \pm 0.80	105.5 \pm 5.50	18.6 \pm 0.1	12.3 \pm 0.3

Coll collenchyma, mid midrib, ue upper epidermis, x ploidy level, mean value \pm standard error, (+) presence, (-) absence^a Inceer and Hayirlioglu-Ayaz (2010)^b Inceer and Beyazoglu (2004)

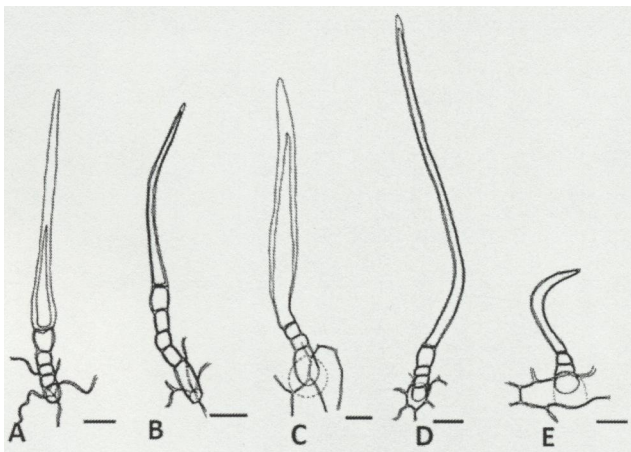


Fig. 2 Trichomes of some representatives of *Matricaria* and *Tripleurospermum*. **a** *M. chamomilla* var. *chamomilla*, **b** *M. chamomilla* var. *recutita*, **c** *T. fissurale*, **d** *T. kotschy*, **e** *T. ziganaense*. Scale bars 40 μ m

One large and two or four medium size vascular bundles are generally found in all taxa (Figs. 3, 4, 5, 6, 7). In addition, accessory vascular bundles are present in all taxa, except for *T. baytopianum*, *T. callosum*, *T. monticolum*, *T. repens*, and *T. rosellum* var. *album* (2x). The midrib vascular bundle is extensive and occupies 50% of the mesophyll region. In addition, it is generally larger in tetraploid taxa and smaller in diploid ones. This bundle is wide in the midrib of *T. callosum*, *T. monticolum*, and the tetraploid cytotype of *T. rosellum* var. *album* (Table 2; Figs. 4, 6, 7). On the other hand, it is narrow in *T. conoclinium* (Fig. 4). Among the taxa, the widest xylem and phloem are present in tetraploid *T. rosellum* var. *album* (Table 2).

Secretory canals (resin ducts) occur in the mesophyll of all accessions in different numbers and sizes. These canals are prominent in all taxa, except in *M. chamomilla* var. *recutita* (Fig. 3).

Multivariate analyses

The UPGMA dissimilarity clustering dendrogram and PCA ordination for all taxa are presented in Fig. 8. Eighteen taxa of *Matricaria* and *Tripleurospermum* fall into two major clusters depending on their ploidy levels. Principal component analysis of the leaf anatomical characters reveals that the first two PC factors accounted for about 74% of the total variance. The first factor accounts for about 59% of the total variance, with the mean thickness of xylem, and the length and breadth of the vascular bundle having the highest positive correlation ($r > 0.91$). The second factor accounts for about 15% of the total variance, with the mean stomatal index of upper epidermis showing the highest positive correlation ($r > 0.67$).

The coefficient of correlation for anatomical parameters of the leaf showed a high r -value for stomatal length

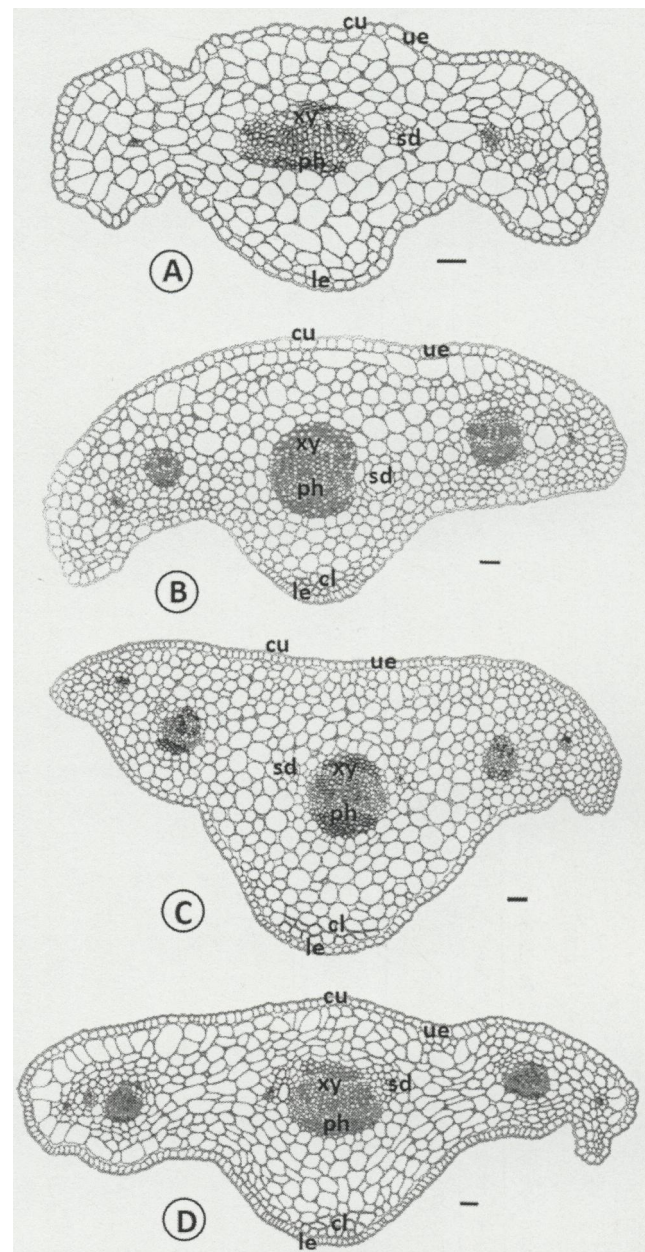


Fig. 3 Transverse sections of *Matricaria* leaves. **a** *M. aurea*, **b** *M. chamomilla* var. *chamomilla*, **c** *M. chamomilla* var. *recutita*, **d** *M. matricarioides*. *cl* collenchyma, *cu* cuticle, *le* lower epidermis, *ph* phloem, *pp* palisade parenchyma, *sd* secretory ducts, *t* simple trichome, *ue* upper epidermis, *xy* xylem. Scale bars 40 μ m

(>0.90). However, lower r -values were found for cuticle thickness, mesophyll breadth (midrib), stomatal index in abaxial surface, and presence or absence of collenchyma (Table 3).

Discussion

Detailed anatomical features of the taxa are presented here for the first time, except for *M. chamomilla* and

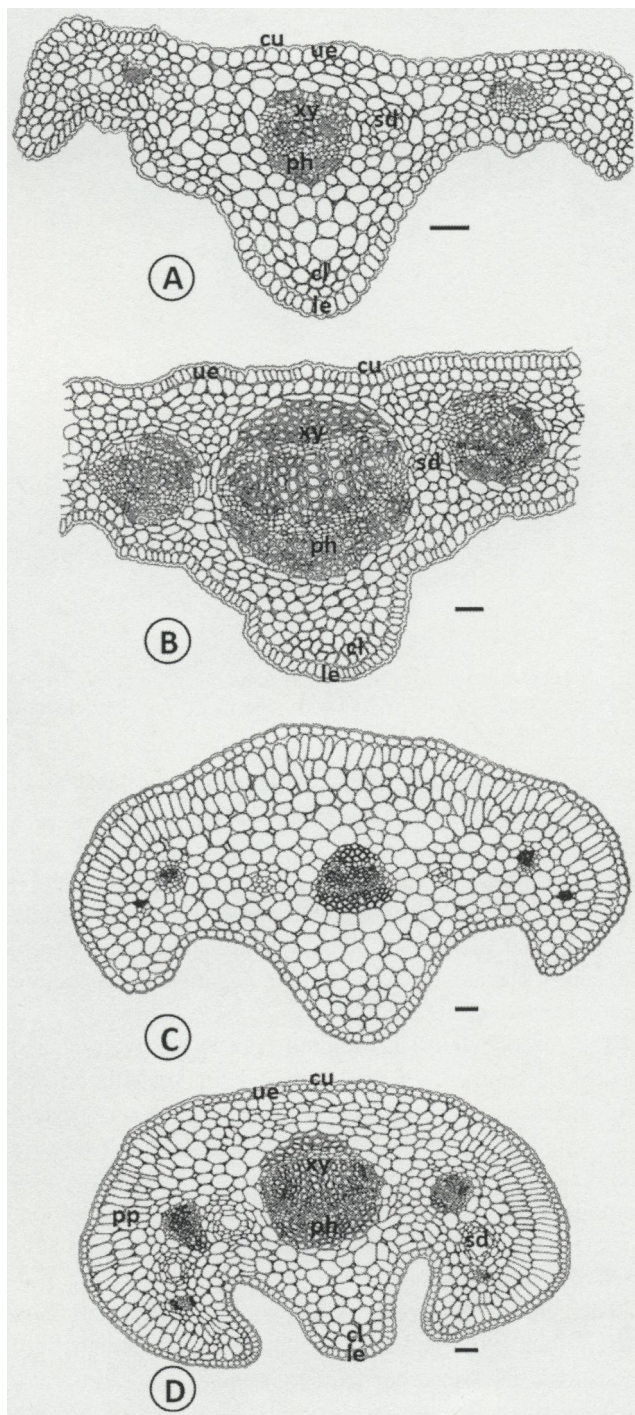


Fig. 4 Transverse sections of *Tripleurospermum* leaves. **a** *T. baytopianum*, **b** *T. callosum*, **c** *T. conoclinium*, **d** *T. corymbosum*. See Fig. 3 for abbreviations. Scale bars 40 μm

T. baytopianum. Napp-Zinn and Eble (1978) for *M. chamomilla* and Uysal (1991) for *T. baytopianum* provided some information on leaf anatomy. The present results are in agreement with these previous reports.

The anatomical structure of the leaves is similar in all taxa studied and is composed of lower and upper epidermis

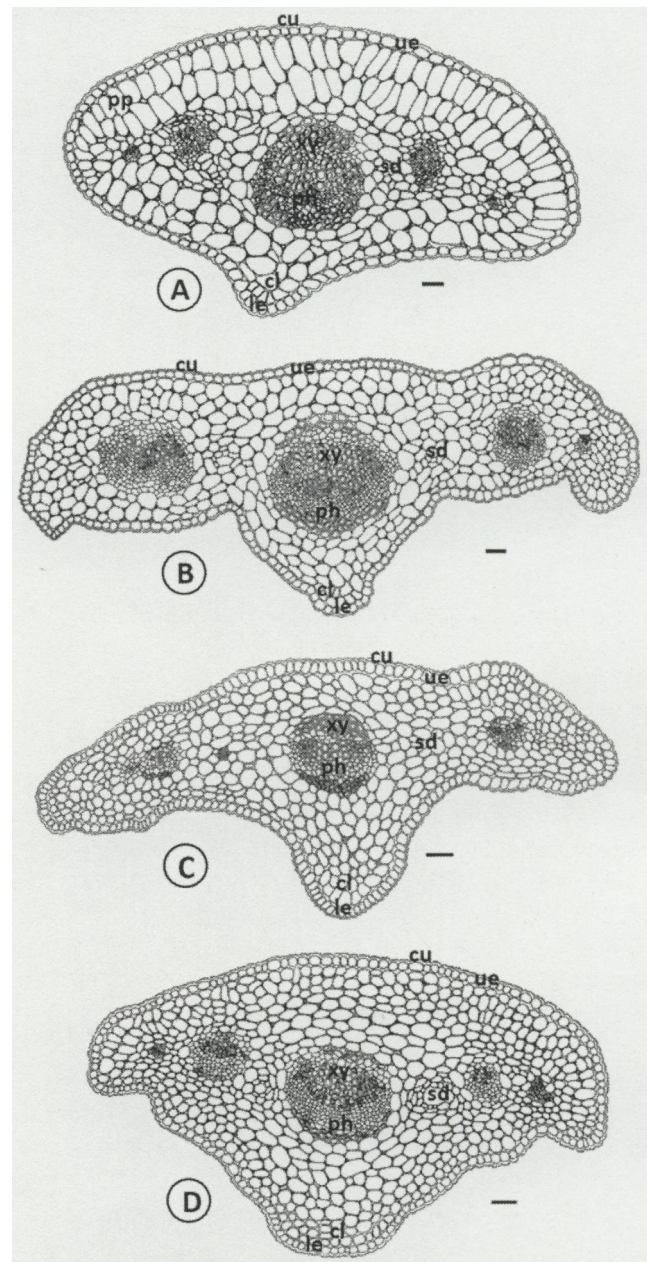


Fig. 5 Transverse sections of *Tripleurospermum* leaves. **a** *T. fissurale*, **b** *T. heterolepis*, **c** *T. hygrophilum*, **d** *T. kotschyi*. See Fig. 3 for abbreviations. Scale bars 40 μm

including anomocytic or anomocytic-anisocytic stomata, parenchymatic mesophyll, and vascular bundle. These characteristics agree with data previously reported by Napp-Zinn and Eble (1978) and Metcalfe and Chalk (1979) for the family Asteraceae. However, the size of some anatomical characters differs based on ploidy level of the taxa without generic delimitation.

There is an extensive overlap in the foliar epidermal characters observed in the representatives of *Matricaria* and *Tripleurospermum* (Fig. 1). The stomatal index of the adaxial side is similar to the abaxial one. The frequency of

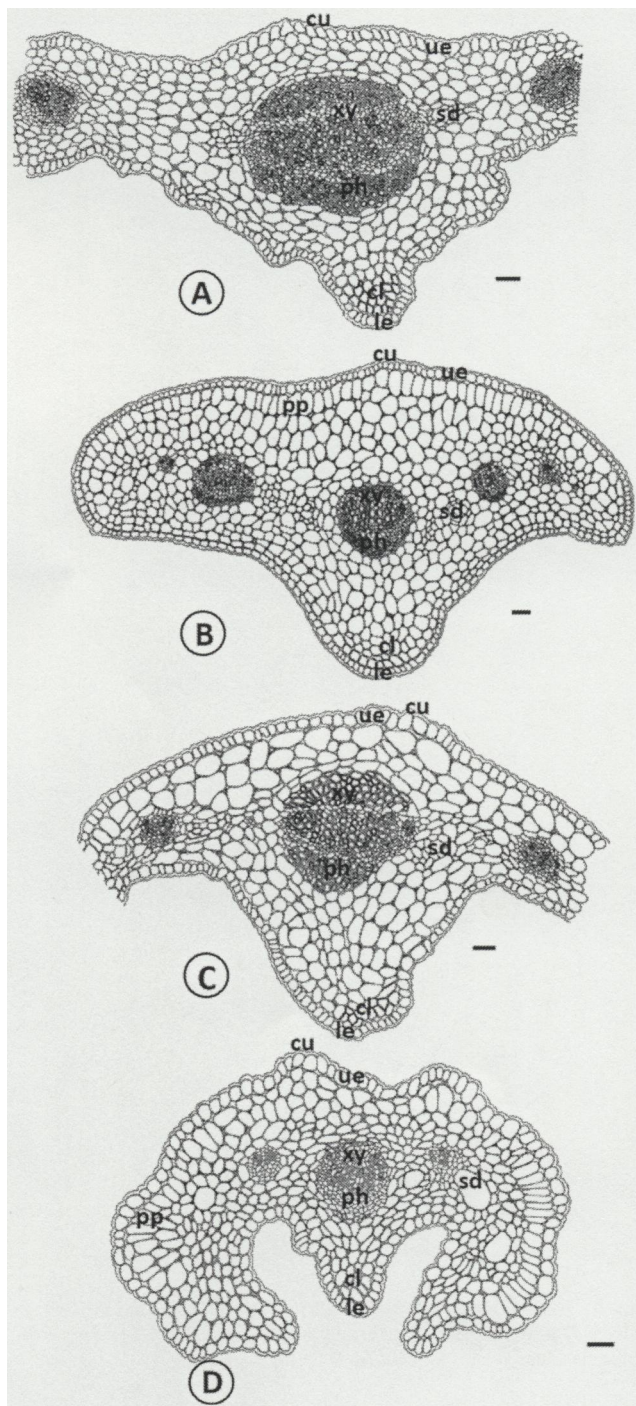


Fig. 6 Transverse sections of *Tripleurospermum* leaves. **a** *T. monticolum*, **b** *T. pichleri*, **c** *T. repens*, **d** *T. rosellum* var. *album* (2x). See Fig. 3 for abbreviations. Scale bars 40 μm

stomata (per 1 mm^2) among the diploid taxa is higher than that of the tetraploid ones (Table 2). It has been reported that diploid species have almost twice as many stomata per square millimeter than do tetraploid species (Sax and Sax 1937). It has also been noted that polyploids have larger stomata than diploids (Stebbins 1971; Seidler-Lozykowska 2003; Inceer and Hayirlioglu-Ayaz 2010).

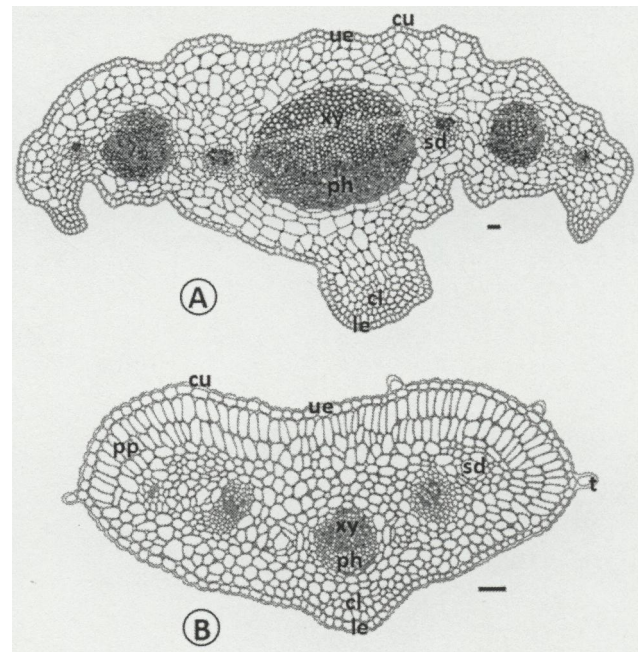


Fig. 7 Transverse sections of *Tripleurospermum* leaves. **a** *T. rosellum* var. *album* (4x), **b** *T. ziganaense*. See Fig. 3 for abbreviations. Scale bars 40 μm

The indumentum of the leaf epidermis is similar in all of the studied taxa. The basic type, uniseriate nonglandular trichomes, was observed in all taxa, except for *T. corymbosum*, which was glabrous. Kadereit and Jeffrey (2007) reported that the leaves of Asteraceae were occasionally glabrous, but glandular and nonglandular trichomes were frequently present, the basic types being biseriate glandular and uniseriate nonglandular.

The mesophyll of the studied taxa is dorsiventral and mostly of similar structure, although its breadth differs. The differences mainly concern the thickness of xylem with length and breadth of the vascular bundle. Metcalfe and Chalk (1979) pointed out that the mesophyll was homogeneous in the family Asteraceae. In addition, Kadereit and Jeffrey (2007) reported that it was usually dorsiventral in this family.

The internal secretory systems, articulated laticifers (laticiferous cells) and schizogenous secretory canals, are widespread in Asteraceae (Kadereit and Jeffrey 2007) and are used for taxonomic evaluations (Appezato-Da-Glória et al. 2008). Schizogenous secretory canals near the phloem were found in all taxa. In particular, together with two varieties of *M. chamomilla*, the cytotypes of *T. rosellum* showed prominent size differences in secretory canals. In addition, the presence of secretory canals in *Matricaria* and *Tripleurospermum* has been reported by some authors (Metcalfe and Chalk 1979; Uysal 1991; Andreucci et al. 2008). According to Tetley (1925) and Williams (1954), since these canals are so close to the phloem, they probably aid the sieve tube in the transfer of organic material.

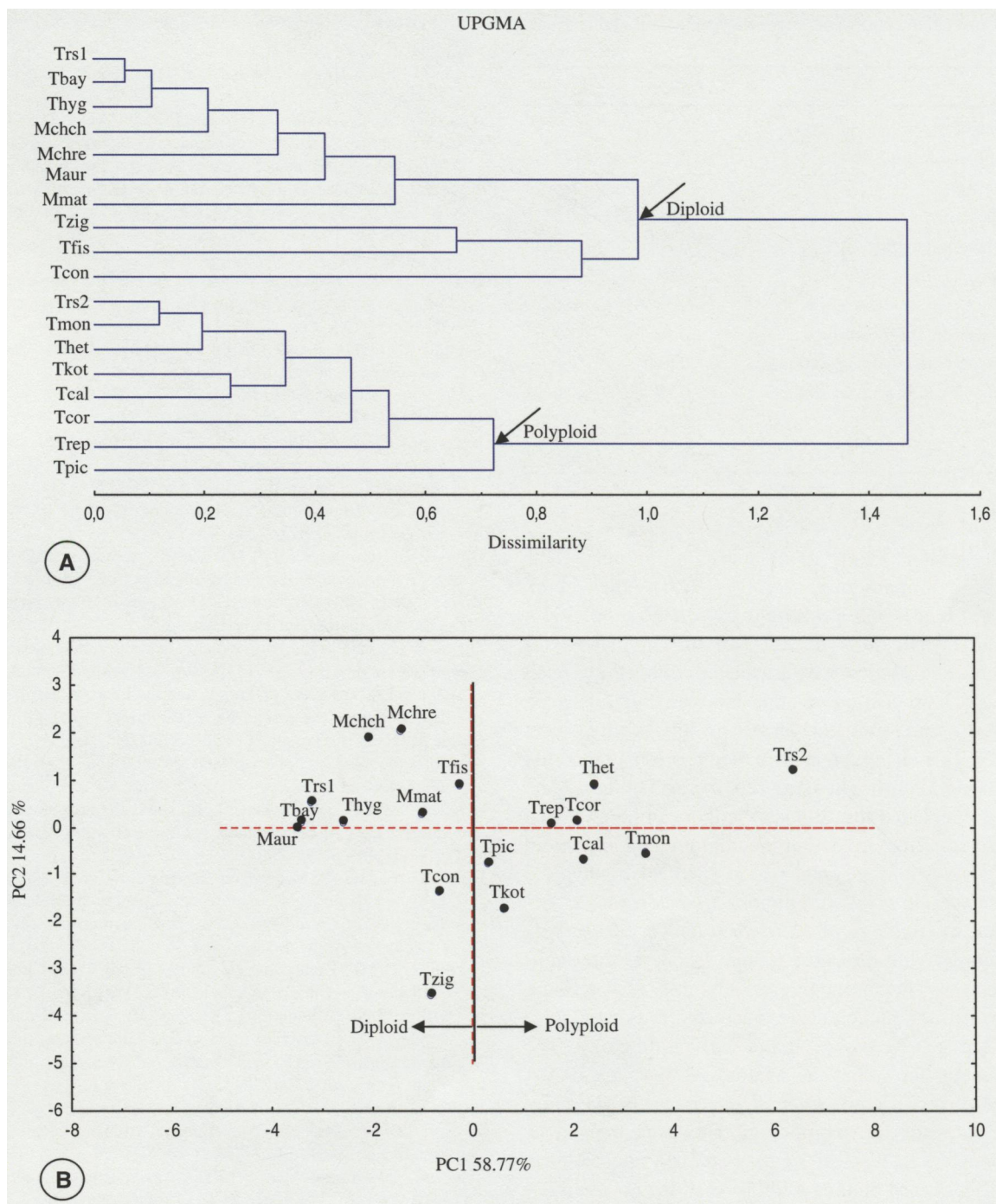


Fig. 8 Clusters of *Matricaria* and *Tripleurospermum* taxa based on leaf anatomical data. **a** UPGMA, **b** PCA. Taxa codes: *Trs1* *Tripleurospermum rosellum* var. *album* (2x), *Trs2* *T. rosellum* var. *album* (4x), *Thyg* *T. hygrophilum*, *Tbay* *T. baytopianum*, *Tzig* *T. ziganaense*, *Tkot* *T. kotschy*, *Tcon* *T. conoclinium*, *Tpic* *T. pichleri*,

Tcal *T. callosum*, *Tcor* *T. corymbosum*, *Thet* *T. heterolepis*, *Trep* *T. repens*, *Tmon* *T. monticulum*, *Tfis* *T. fissurale*, *Mchch* *Matricaria chamomilla* var. *chamomilla*, *Mchre* *M. chamomilla* var. *recutita*, *Maur* *M. aurea*, *Mmat* *M. matricarioides*

The variation in leaf structure is correlated to ploidy levels of the taxa (Table 3). The UPGMA reveals that diploid and polyploid taxa are mainly found in two major clusters (Fig. 8). In addition, PCA shows that some anatomical characters such as thickness of xylem, dimensions

of vascular bundle, and stomatal index of the adaxial epidermis seem to be important and explain most of the total variation among the taxa.

Polyploidy is considered by many authors as an important evolutionary mechanism in plants (Bretagnolle

Table 3 Correlation coefficients between ploidy level and leaf anatomical characters

Character	<i>r</i>	<i>P</i> value
Cuticle thickness	0.24	NS
Mesophyll breadth (midrib)	0.45	NS
Xylem thickness	0.72	<0.001
Phloem thickness	0.59	<0.01
Vascular bundle (length)	0.70	<0.001
Vascular bundle (breadth)	0.68	<0.002
Stomatal index in adaxial surface	-0.54	<0.02
Stomatal index in abaxial surface	-0.30	NS
Number of stomata in adaxial surface	-0.89	<0.0001
Number of stomata in abaxial surface	-0.79	<0.0001
Stomatal length	0.92	0.0000
Presence or absence of collenchyma	0.31	NS

et al. 1998; Soltis et al. 2004) and some groups of the Anthemideae in particular (Solbrig 1977; Vallès et al. 2001, 2005; Inceer and Beyazoglu 2004; Guo et al. 2005; Inceer and Hayirlioglu-Ayaz 2007, 2010). The majority of the anatomical variation was associated with ploidy level (Table 3). A high positive relationship was found between ploidy level and stomatal length in the studied taxa ($r = 0.92$). This result agrees with data previously reported by Inceer and Hayirlioglu-Ayaz (2010) for *Tripleurospermum*. It is concluded that stomatal length can be used as a taxonomic criterion to determine diploid and polyploid taxa in *Tripleurospermum* and also to distinguish polyploid *Tripleurospermum* taxa from diploid *Matricaria* taxa.

The leaf morphology of *Matricaria* and *Tripleurospermum* is similar with dissected foliage of one to three pinatisect leaves. This morphological similarity is also found in other genera of Anthemideae. Therefore, it is difficult to separate the genera based on the leaf morphology. The present results show that the anatomical structure of the leaf in *Matricaria* and *Tripleurospermum* is basically the same, as is the leaf morphology. However, the size of some characters in the leaf such as stomata and vascular bundle differs based on the ploidy level of the taxa without generic delimitation. We suggest that these characters can be used as an anatomical marker for distinguishing diploid from polyploid taxa, but cuticle thickness, mesophyll breadth (midrib), stomatal index in abaxial epidermis, and presence or absence of collenchyma can not.

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