

# Micromorphological and Anatomical Characteristics of the Genus *Chrysophthalmum* Schultz Bip. (Asteraceae) Growing in Turkey

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**Abstract** The genus *Chrysophthalmum* (Asteraceae) is represented by five species in the world. Three species of this genus are naturally grown in Turkey. The species growing in Turkey are *C. montanum* (DC.) Boiss., *C. dichotomum* Boiss. and Heldr. and *C. gueneri* Aytac and Anderb. Except *C. montanum*; the other species are endemic for Turkey. In the present study, micromorphological and anatomical characteristics of these species by using light microscopy (LM) and scanning electron microscopy (SEM) have been comparatively presented for the first time. The micromorphological studies are related to trichomes and cypsela. In anatomical studies, cross-sections of the stem, and leaf have been examined. In addition, to show stomatal distribution and anatomy on adaxial and abaxial leaves, surface sections of the leaves have been taken and stomatal index calculated. The features of stems, leaves, trichomes and cypsela have been found to be significant to

distinguish the species. Mesophyll structure, number of vascular bundles in midrib, presence or absence of sclerenchymatous fibers in leaf, trichome types, presence or absence of pappus in cypsela, presence or absence of hair on cypsela surface are important diagnostic characters. A diagnostic key based on combined leaf, trichome and cypsela characteristics of the *Chrysophthalmum* species has been given.

**Keywords** Anatomy · Asteraceae · *Chrysophthalmum* · Cypsela · Micromorphology · Trichome

## Introduction

Asteraceae (Compositae) is one of the largest angiosperm families and according to recent classification it is divided into four subfamilies and 17 tribes. Within the family, a total of 1,535 genera with 26,000 species have been recorded [1, 2]. Asteraceae is widely distributed within diverse regions ranging from Southwest of US, Mexico, Southern Brazil, South Africa, Middle and Southwest Asia as well as Australia. South America is accepted as the place of origin of the family [3–5]. Asteraceae is represented by 1,209 species in the Turkish Flora. 447 of these species are endemic for Turkey with endemism ratio as 37 %. With a total number of 134 genera, this family constitutes the second largest family of Turkish flora [2, 6–8].

The genus *Chrysophthalmum* Schulz Bip. is represented by five species on earth and three of them are naturally grown in Turkey. These species are *C. montanum* (DC.) Boiss, *C. dichotomum* Boiss. and Heldr. and *C. gueneri* Aytac and Anderb. Except *C. montanum* other taxa are endemic for Turkey [6, 7, 9–11].

Metcalf and Chalk studied the anatomy of the family Asteraceae and determined the diagnostic anatomical

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characteristics as epidermal cell type, stoma type, presence of latex, and the arrangement of the sclerenchymatous cells around the vascular bundles of the leaves [12]. Taxonomic studies have been elaborated during the last few years [13–18].

The micromorphological and anatomical characteristics of genus *Chrysophthalmum* have not been studied so far. The present study aims to give a detailed account of the micromorphological and anatomical features of these species.

**Table 1** Specimens used for micromorphological and anatomical studies and collected localities

Species	Collection areas and collector's number
<i>C. montanum</i> (DC.) Boiss.	Turkey, B7 Malatya, Karagöz village, rocky slopes, 1,595 m, 20.07.2012, Paksoy 1677; C5 Niğde: Ulukışla, between Maden village and Ali hoca, rock and limestone cliffs, 1,350 m, 11.07.2011, Paksoy 1301; C5 Adana, between Saimbeyli and Ümmet Uşağı village, rocky cracks, 700 m, 22.07.2012, Paksoy 1685
<i>C. dichotomum</i> Boiss. and Heldr.	Turkey, C3 Antalya, Akseki village, around Moru, dam and shrubby valley beds, 1,200 m, 06.08.2011, Paksoy 1315; C3 Antalya, Alanya, Akçatı vicinity, rocky slopes, 750 m, 25.07.2012, Paksoy 1703
<i>C. gueneri</i> Aytac and Anderb.	Turkey, C4 Konya: between Taşkent and Alanya, Gevne valley, around Cirlasun bridge, in <i>Pinus nigra</i> forest clearing, 12.07.2011, Paksoy 1302

**Table 2** Stem anatomical characters of *Chrysophthalmum* genus

Taxa	Cortex				Interfascicular region	Phloem region
	Surface of stem	Collenchyma	Parenchyma	Endodermis		
<i>C. montanum</i>	2–3 grooved	–	4–6	1–2	2–4 row	2–5
<i>C. dichotomum</i>	Straight	1–2	3–5	1–2	3–4 row	3–5
<i>C. gueneri</i>	Straight	–	3–5	1–2	3–4 row	2–5

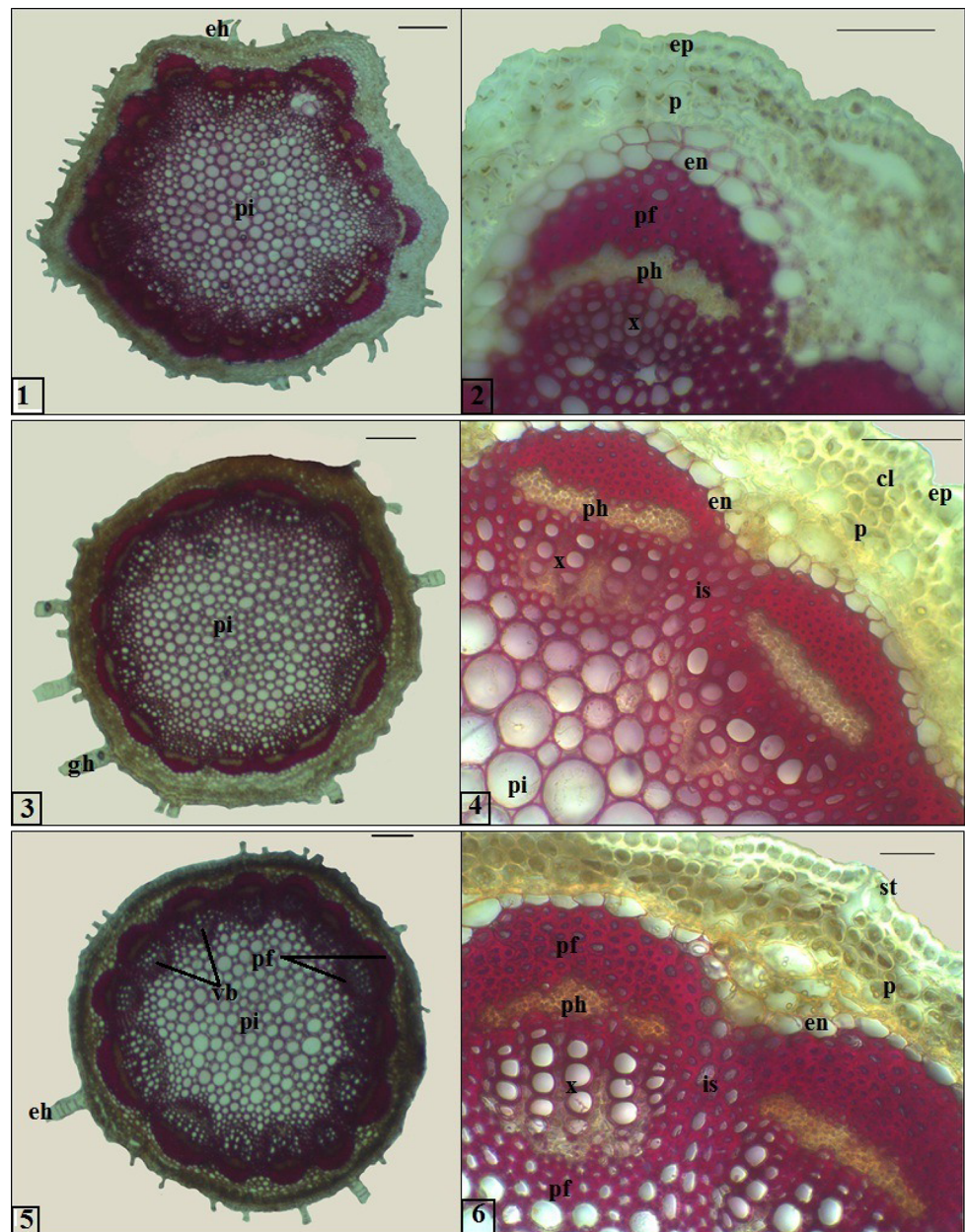
**Table 3** Leaf anatomical characters of *Chrysophthalmum* genus (Ad.: adaxial; Ab.: abaxial)

Taxa	Mesophyll type	Palisade layer	Spongy layer	Middle vascular bundle						
				Collenchyma		Parenchyma		Bundle sheat	Xylem fibers	Phloem fibers
				Ad.	Ab.	Ad.	Ab.			
<i>C. montanum</i>	Equifacial	1–2	2–4	1–2	2–3	2–4	2–5	Absent	Present	Present
<i>C. dichotomum</i>	Bifacial	1–2	4–6	–	1–2	3–5	5–7	Absent	Rare	Absent
<i>C. gueneri</i>	Bifacial	1–2	4–6	–	–	4–6	3–6	Present	Absent	Present

**Table 4** Characteristics of the leaf epidermis of *Chrysophthalmum* genus under light microscopy

Characters	<i>C. montanum</i>		<i>C. dichotomum</i>		<i>C. gueneri</i>	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Anticlinal cell wall	Undulate	Undulate	Undulate	Undulate	Undulate	Undulate
Stomata type	Anomocytic	Anomocytic	Anomocytic	Anomocytic	Anomocytic	Anomocytic
	–	Anisocytic	Anisocytic	Anisocytic	Anisocytic	Anisocytic
Shape of epidermis cells	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular
Stomata length ( $\mu$ )	19 $\pm$ 1.4	23 $\pm$ 1.6	21.3 $\pm$ 1.2	22.2 $\pm$ 0.9	22.6 $\pm$ 2.4	19.7 $\pm$ 2.3
Stomata width ( $\mu$ )	13.9 $\pm$ 1.6	17.5 $\pm$ 1.5	13.5 $\pm$ 1.2	14.9 $\pm$ 0.7	15.1 $\pm$ 1.4	14.5 $\pm$ 1.4
Number of stomata (1 mm <sup>2</sup> )	176 $\pm$ 12	134 $\pm$ 17	200 $\pm$ 21	167 $\pm$ 18	200 $\pm$ 21	272 $\pm$ 8
Number of epidermis cells	275 $\pm$ 23	320 $\pm$ 23	219 $\pm$ 16	370 $\pm$ 30	282 $\pm$ 22	396 $\pm$ 15
Stomata index	39.02	29.5	47.7	31.09	41.4	40.7
Stomata index ratio	1.3		1.5		1.01	

**Fig. 1** Stem structures in cross-section of *Chrysophthalmum* species. 1–2. *C. montanum*, 3–4. *C. dichotomum*, 5–6. *C. gueneri*. cu: cuticle, eh: eglandular hair, gh: glandular hair, ep: epidermal cell, st: stomata, cl: collenchyma, p: parenchyma, en: endodermis, pf: phloem fibers, ph: phloem, x: xylem, is: interfascicular cell, pi: pith. (Scale bar: 20  $\mu$ )



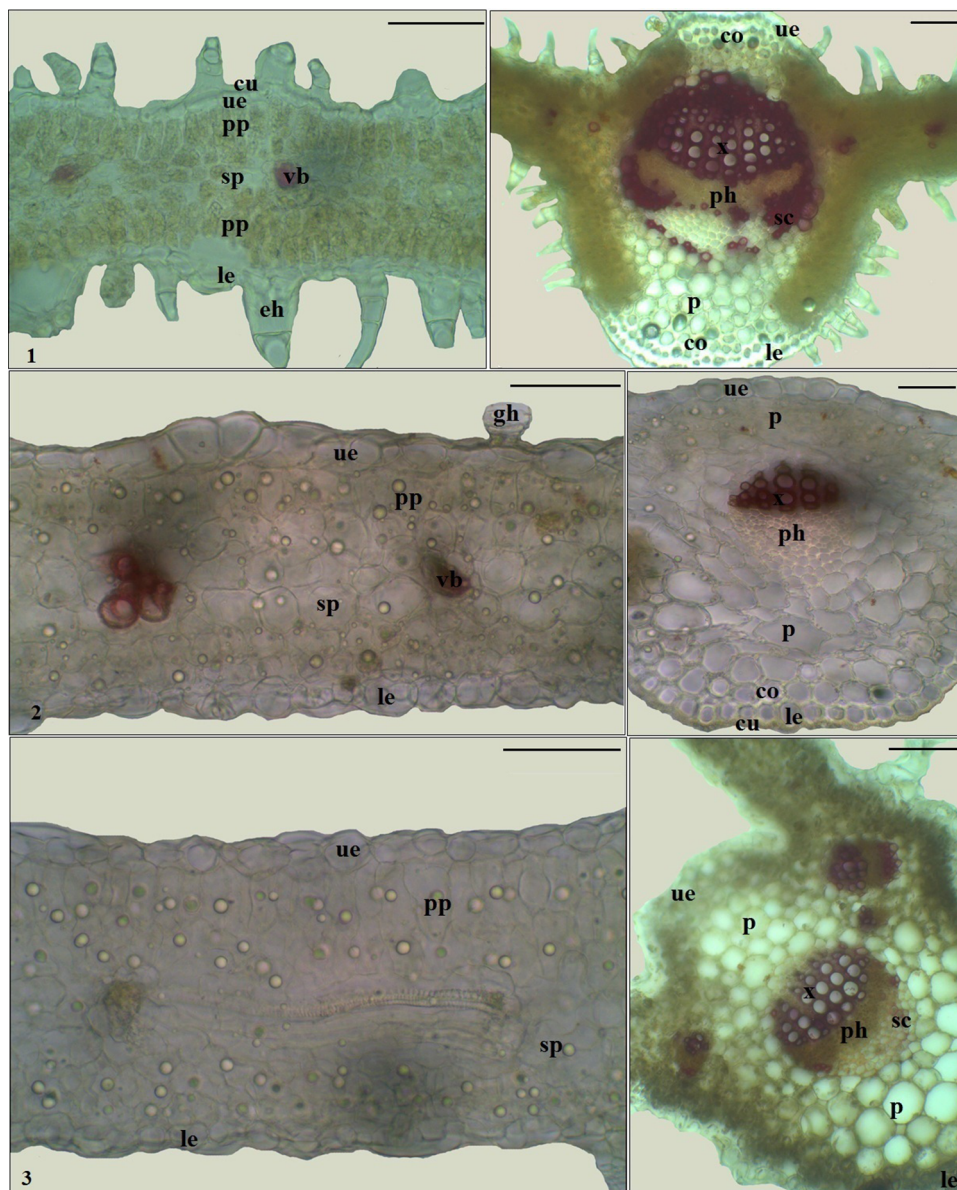
**Material and Methods**

Plant specimens were collected from different localities in Turkey and have been stored in Tunceli University Herbarium (Table 1). Anatomical studies were carried out on specimens kept in 70 % alcohol. Cross-sections of stem and leaves were stained with Phloroglucinol–HCl solutions [19] and the chlorophyll in leaves was removed with chloral hydrate.

Stomatal density on abaxial and adaxial surfaces of the leaves has been counted under a light microscope. Stomatal index has been calculated according to the method of

Meidner and Mansfield [20]. Stomatal terminology (stomatal types, arrangement of guard and subsidiary cells) and the leaf epidermal terminology are based on the classification proposed by Dilcher [21] and Wilkinson [22] respectively. Measurements and photographs are taken using Olympus BX 51 and Nikon Eclipse E600 binocular light microscopes. Trichome and cypselae micromorphology is studied by Tabletop scanning electron microscopy (SEM). For SEM, small pieces of leaves and stem with cypselae were fixed on aluminum stubs using double-sided adhesive. The SEM micrographs were taken in a NeoScope JCM–5000 at an accelerating voltage of 10 kV.

**Fig. 2** Mesophyll tissue (left) and midrib (right) structures in leaf cross-section of *Chrysophthalmum* species. 1. *C. montanum*, 2. *C. dichotomum*, 3. *C. gueneri*. cu: cuticle, gh: eglandular hair, eh: eglandular hair, ue: upper epidermis (adaxial surface), pp: palisade parenchyma, sp: spongy parenchyma, vb: vascular bundle, x: xylem, ph: phloem, sc: sclerenchyma, le: lower epidermis (abaxial surface). (Scale bar: 50  $\mu$ )



## Results and Discussion

Comparative anatomical characters of stems and leaves of *Chrysophthalmum* species are given in Tables 2 and 3 respectively. In addition, the features of the leaf epidermis of *Chrysophthalmum* species are shown in Table 4.

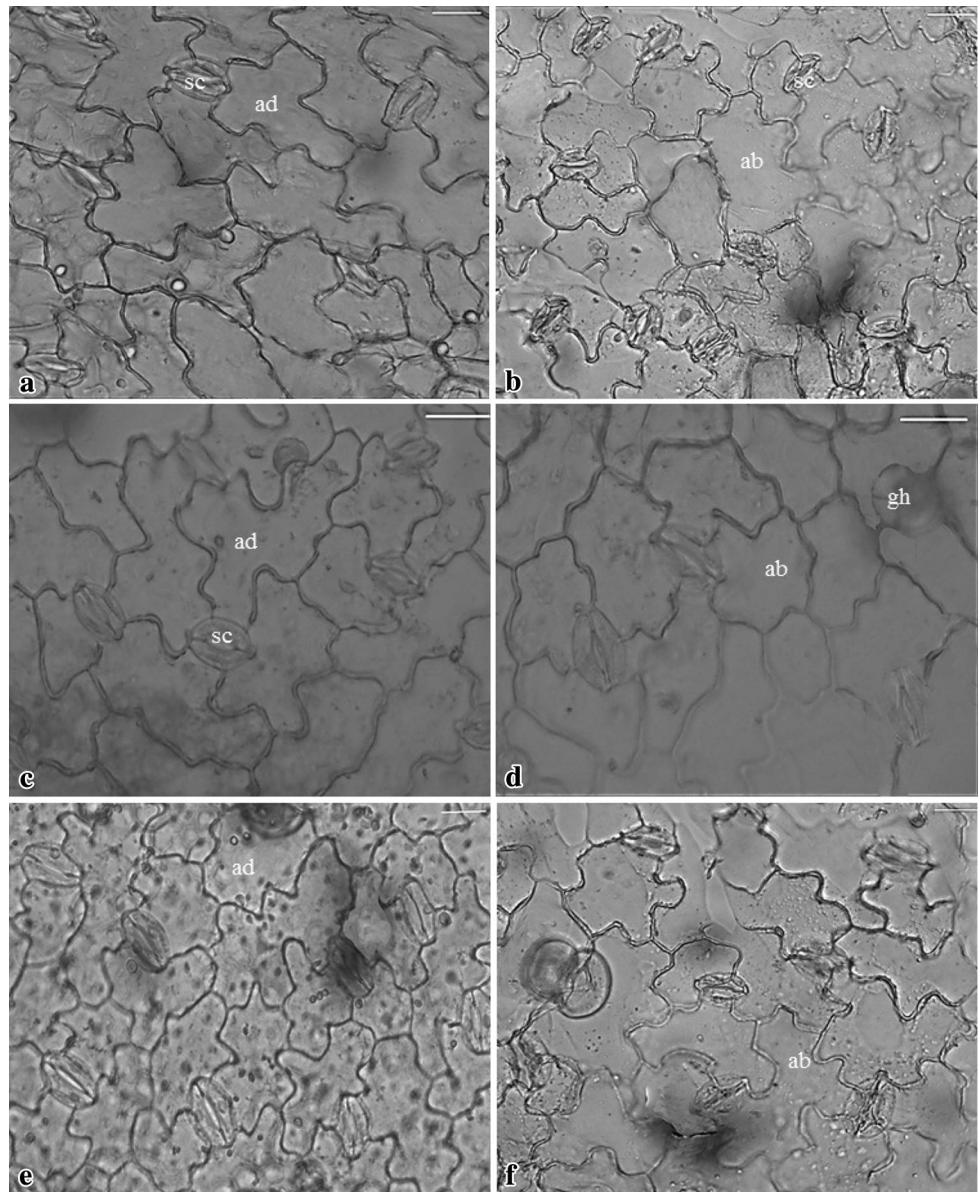
### General Anatomical Characters of the Genus

#### Stem Anatomy

In the transverse sections of the stem, the single-layered epidermis is the outermost layer which is covered by a thin layer of cuticle (Fig. 1). The epidermis contains eglandular (bicellular, multicellular) and glandular (uniserial,

biseriate) trichomes (Figs. 4, 5). Epidermis shows stomata which are of epistomatic and anomocytic type. The cortex consists of collenchyma, parenchyma and endodermis. Underneath the epidermis, collenchymas tissue (1–2 layered) may be present or absent. The parenchyma tissue consists of oval or orbicular chlorenchyma cells arranged in 3–6 layers. Underneath the parenchyma tissue, there are 1–2 layered, large, oval or rectangular endodermal cells. The number of vascular bundles are arranged in a ring of 13–24 (Fig. 1). Sclerenchymatous interfascicular region is located in between vascular bundles. The phloem, with sclerenchymatous fibers, measures 11–46  $\mu$ m. It shows 2–5 layers, and consists of irregular and squashed cells. Cambium is not distinguishable. The pith is wide and consists of polygonal or orbicular parenchymatic cells.

**Fig. 3** Leaf surface-section of *Chrysophthalmum* species. Adaxial surface (a, c, e) and abaxial surface (b, d, f). *C. montanum* (a, b), *C. dichotomum* (c, d), *C. gueneri* (e, f). ad: adaxial epidermal cell, ab: abaxial epidermal cell, gh: glandular hair, sc: stomata cell (Scale bar: 20  $\mu$ )



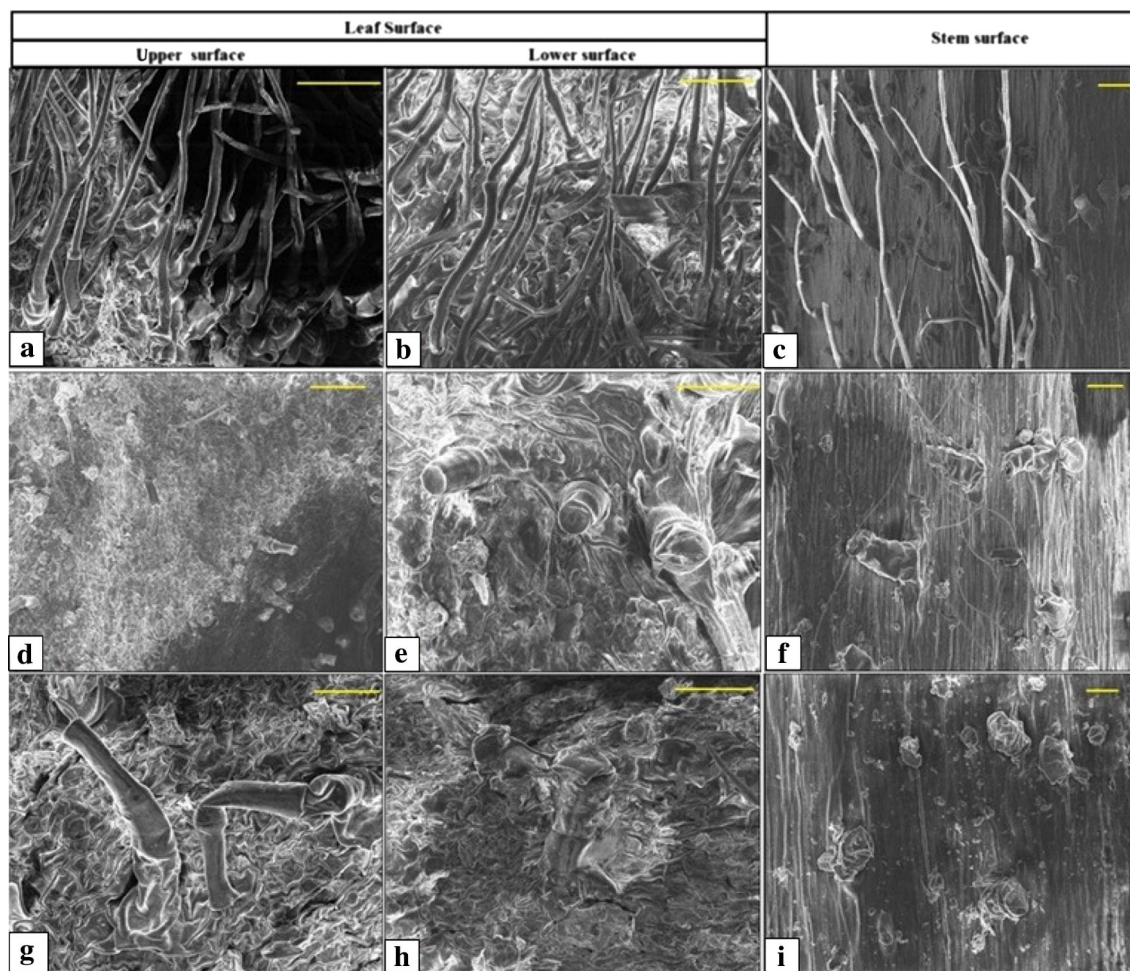
### Leaf Anatomy

The transverse section of the leaf shows a thin cuticle on the upper and lower epidermis (Fig. 2). The epidermal cells are isodiametric and rectangular, oval or cuboidal in shape. Surface of epidermis is covered with eglandular (1–4 cells) and glandular (uniseriate and biseriate) hair (Fig. 4, 5). Amphistomatic type of stomata are present on both surfaces of the leaf. In surface section, the epidermal cell walls are undulate and stomata type is Ranunculaceous (Anomocytic) or rarely Cruciferous (Anisocytic) type (Fig. 3). The stomatal index is 31–47 on upper surface and 29–47 on lower surface. The stomatal index ratio is between 1.01 and 1.5. Mesophyll consists of 1–2 layered palisade and 2–6 layered spongy parenchyma cells.

Mesophyll is bifacial and equifacial (Fig. 2). Vascular bundles may or may not be surrounded by parenchymatic bundle sheaths. Sclerenchymatous tissue on the xylem and phloem may or may not be present. The xylem faces the upper surface while phloem faces the lower epidermis.

### General Cypsela Characters

Cypsela narrowly obovate, obovate–oblongoid or narrowly ellipsoidal to cylindrical, 1.2–2.5  $\times$  0.14–0.38 mm, pale brown to dark brown, glabrous or hairy. Pappus is present or absent. Surface cells of cypsela are short or elongate and prismatic. Carpodium appears as a circular ring or slightly angular (Fig. 6).



**Fig. 4** Stem and leaf eglandular (a–c) and glandular trichomes (d–i) of *C. montanum* (a–c), *C. dichotomum* (d–f), *C. gueneri* (g–i)

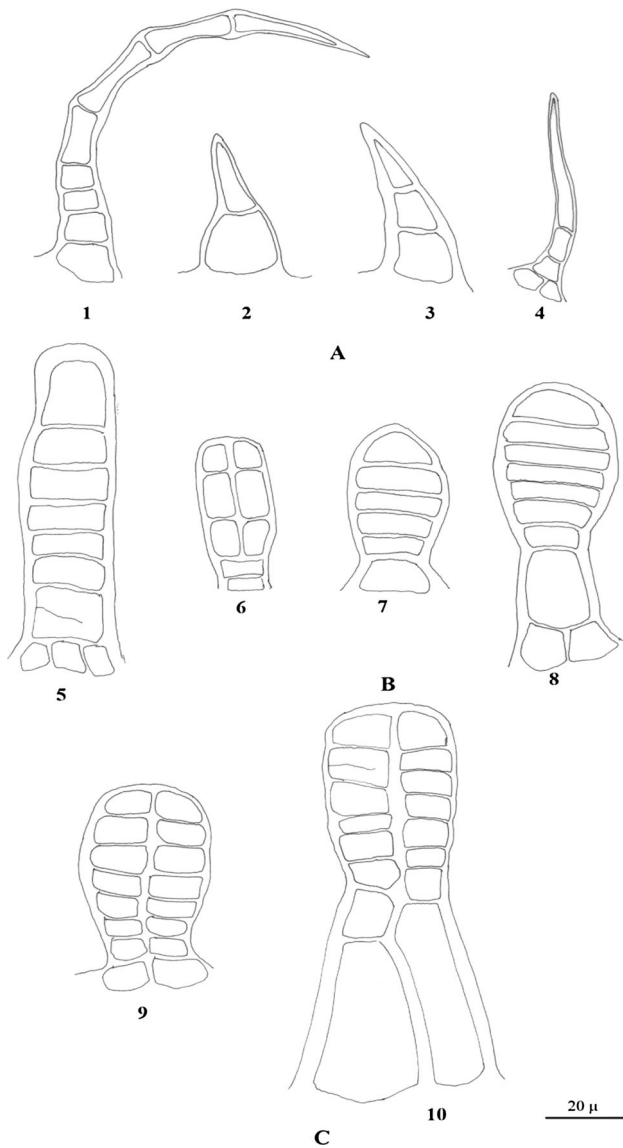
#### General Trichome Characters

Two different types of trichomes have been observed on the stem and leaves of *Chrysophthalmum* species (Figs. 1, 2, 3, 4). They are non-glandular and glandular. Non-glandular trichomes are bicellular or multicellular, acicular or curved, mostly having one to three cells, arranged in a single row (Figs. 4a–c, 5a). Glandular trichomes can be divided into 4 types: 1. Sessile uniseriate, 2. Stalked uniseriate 3. Sessile biseriate 4. Stalked biseriate. Sessile uniseriate trichomes are rod shaped (Fig. 4e, f, 5-B5) or capitate (Fig. 5-B7) and composed of a unicellular base, and a multicellular head. Stalked uniseriate trichomes are capitate and composed of a uni or bicellular base, unicellular stalk and a multicellular capitate head (Fig. 5-B8). Sessile biseriate trichomes are rod shaped or capitate, composed of a unicellular base, and two rowed multicellular head (Fig. 5-B6, C9). Stalked biseriate trichomes are capitate and composed of a bicellular base, two rowed-bicellular stalk and two rowed multicellular capitate head (Fig. 5-C10).

*Chrysophthalmum* species shows the typical features of the family Asteraceae [12]. Anatomically, important characters for identification of *Chrysophthalmum* species are the number of vascular bundles in stem, surface of stem (grooved or straight), mesophyll structure, the number of vascular bundles of midrib in leaves, presence or absence of bundle sheath surrounding vascular bundles in leaves, presence or absence of xylem and phloem fibers in leaves, the number of cortical parenchyma layer, presence or absence of sclerenchyma, mesophyll structure, and epidermal surface.

Mesophyll type is anatomically significant for *Chrysophthalmum* species. In *C. dichotomum* and *C. gueneri* it is bifacial while in *C. montanum* equifacial (Fig. 2). The structure of midrib of leaf can be used as a very significant character for distinguishing the *Chrysophthalmum* species. *C. gueneri* has single large vascular bundle in the middle region and two small bundles whereas *C. montanum* and *C. dichotomum* have only one large bundle in the middle of the midrib (Fig. 2).

According to Metcalfe and Chalk [12], laticiferous channels are commonly observed in the leaves of species

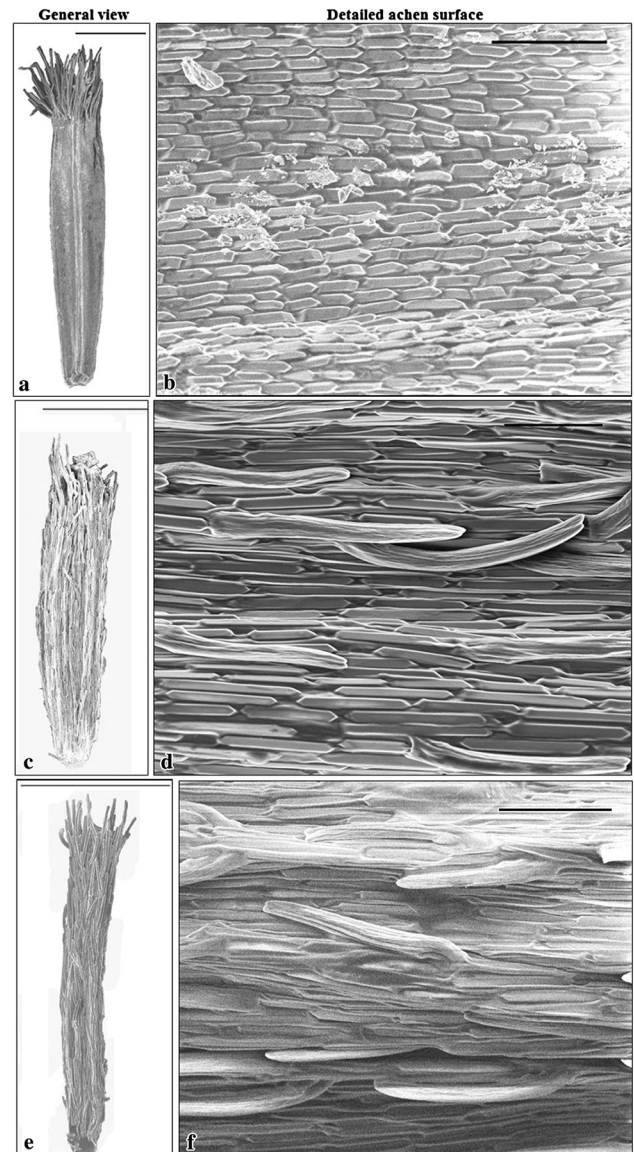


**Fig. 5** Illustration of stem and leaf eglanular (a) and glandular trichomes (b, c). *C. montanum* (A2, A3, A4, B6), *C. dichotomum* (A1, B5, B7), *C. gueneri* (B8, B9, B10)

belonging to the Asteraceae. Latex channels are very important in the comparative anatomical studies and their contents and distribution differ among the members of Asteraceae [23]. In the present study, latex channels have not been observed in stem and leaves.

According to Metcalfe and Chalk [12], the family of Asteraceae shows both anomocytic and anisocytic stomata which have been encountered in the genus *Chrysophthalmum* (Fig. 3).

Trichome types are considered as important accessory characters, especially at lower taxonomic level [24–31]. The non-glandular and glandular hair as a micro character of leaves could be occasionally used in the classification, especially at generic and specific levels [32, 33]. Together



**Fig. 6** Cypselas micromorphology of *Chrysophthalmum* species. *C. montanum* a, b, *C. dichotomum* c, d, *C. gueneri* e, f. Scale bar: a, c, e 500 μ; b, d, f 50 μ

with other characters, hair are important in taxonomic and phylogenetic studies of Asteraceae [34]. In the present study, trichomes appear as important accessory characters for identification of species. Non glandular trichomes have been observed on stem and leaf while glandular trichomes have not been observed in all studied taxa. Stalked biseriate trichomes have been found only *C. gueneri* (Fig. 4g, i, 5-C10).

The importance of cypselas for the phylogeny and classification of the family is underlined in several recent works [34]. In the present study, cypselas appears to be an important accessory character for identification of species (Fig. 6). Pappus is also an important character for

distinguishing the taxa as it is present in *C. montanum* while absent in *C. dichotomum* and *C. gueneri* (Fig. 6).

A diagnostic key is presented below.

1. Mesophyll type equifacial, fibers apparently present within the xylem. Cypsela glabrous, pappus present ..... *C. montanum*  
Mesophyll type bifacial, fibers rare or absent from the xylem. Cypsela hairy, pappus absent ..... 2.
2. Biseriate glandular trichome present or absent within middle vascular bundle in leaves, phloem tissue not includes fibers. Cypsela obovate-oblongoid, pale brown, 0.3–0.33 mm width ..... *C. dichotomum*
3. Biseriate glandular trichome present or absent within middle vascular bundle in leaves, phloem tissue includes fibers. Cypsela narrowly elipsoidal to cylindrical, brown, 0.14–0.15 mm width ..... *C. gueneri*

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