

Anatomical and Palynological Studies on *Chaerophyllum astraintiae* and *C. aureum* in Turkey

Gülden YILMAZ¹, Mehmet TEKIN²

¹Trakya University, Faculty of Science, Department of Biology, Balkan Campus, 22030, Edirne/Turkey

²Cumhuriyet University, Faculty of Science, Department of Biology, 58140, Sivas, Turkey;
guldennyilmaz2009@yahoo.com (*corresponding author), mtekin2280@gmail.com

Abstract

In this study, anatomical and palynological properties of *Chaerophyllum astraintiae* Boiss. & Bal. and *C. aureum* L. were determined for the first time. Cross sections of roots, stems, stem leaves and fruits of both species were examined. The leaves are amphistomatic and mesophyll is bifacial. The fruit has two vittae on commissural face and four vittae on lateral and dorsal faces in both species. The palynological studies revealed that, *C. astraintiae* and *C. aureum* have tricolporate pollen type and perforate exine ornamentation under light microscope. Ratio of polar axis to equatorial axis is 2.15 in *C. astraintiae* and 2.05 in *C. aureum*. Thus, pollen shape is perprolate for both of the species.

Keywords: anatomy, *Chaerophyllum*, palynology, Turkey

Introduction

Apiaceae (or Umbelliferae) commonly known as carrot or parsley family is a family of mostly aromatic plants with hollow stems. It is one of the largest plant family in the world, with 446 genus and 3540 species (Simpson, 2006). Apiaceae is the 8th largest family in Turkey with approximately 455 species among which 33% are endemic (Davis *et al.*, 1988; Guner *et al.*, 2000; Ozhatay *et al.*, 2008-2009, 2011). Apiaceae is solidly placed within the Apiales order in the APG III classification system. Traditionally groups within the family have been delimited largely based on fruit morphology (Stevens, 2001). The family is rich in secondary metabolites and embodies numerous genera of high economic and medicinal value yielding flavonoids, essential oils, terpenes and coumarins. It is well-known that occurrence of oleoresins and essential oils is a characteristic feature of the family. *Chaerophyllum* the largest genus in the subtribe Scandicinae, comprising over 30 species (Downie *et al.*, 2000). It is native to Europe, Asia, North America and northern Africa. Based on a DIVA analysis Chung *et al.* (2005) considered that Eurasia was the ancestral area of the Scandicinae. All *Chaerophyllum* species except *C. procumbens* and *C. tainturieri*, are native to Eurasia and North Africa. Spalik and Downie (2001) suggested four sections in *Chaerophyllum* based on ITS internal transcribed spacer and morphological data. The genus is comprised of about 45 species in the world, 35 species in Asia and 16 species in Turkey (Pimenov and Leonov, 2004) and 4 of these species (*C. byzantium* Boiss., *C. aureum* L., *C. bulbosum* L. and *C. temulum* L.) are grown in

European part of Turkey (Hedge and Lamond, 1972). In this context, *C. aureum* is widely spread in Turkey, while *C. astraintiae* is locally distributed especially in Rize and Artvin province in Turkey. In recent years, a number of studies have been performed on *Chaerophyllum* L. with particular reference to composition of essential oils (Razavi and Nejad-Ebrahimi, 2010; Chizzola, 2009; Nematollahi *et al.*, 2005; Baser *et al.*, 2000), antimicrobial activity of essential oils (Lakusic *et al.*, 2009), antioxidant compounds (Dall'Acqua and Innocenti, 2004) and flavonoid glycosides (Gonnet, 1985). However, there is not enough study on anatomical and palynological data of this genus. Anatomical structure of root, stem, stem leaves and fruit are very important as biochemical composition of the genus *Chaerophyllum* L. The aim of this study is to observe the anatomical and palynological structures of *C. astraintiae* and *C. aureum*.

Material and methods

The specimens used in this study were collected from natural habitats in Rize and Artvin province (Turkey). Localities of *C. astraintiae*: Artvin province, Borcka, Karcal Mountain, near Adagul upland, N 41° 20' 32.7" E 41° 58' 18.3", 2915 m, 04.08.2010, M. Tekin 1035; Artvin province, Karcal mountain, N 41° 21' 29.1" E 41° 58' 08.7", 2557 m, 02.09.2010, M. Tekin 1050. Localities of *C. aureum*: Rize province, İkizdere to Cimil upland, near stream, N 40° 44' 02.9" E 40° 47' 45.9", 2062 m, 01.08.2010, M. Tekin 1018; Artvin province, Şavşat, Karagöl, Cinoba site, alpine meadow, N 41° 19' 26.6" E 42° 29' 56.4", 1763 m,

02.08.2010, M. Tekin 1019. Some specimens were prepared as herbarium materials and deposited in Cumhuriyet University Herbarium (CUFH).

For anatomical studies, the root, stem, stem leaf and fruit of the some specimens were fixed and conserved in 70% ethyl alcohol. Hand sections were made with a razor blade and sections were stained with Alcian blue (Sigma) for pectic substances, Safranin (Sigma) for lignin in the rate of 3/2 (Yılmaz and Dane, 2013). Sections were left in dye about 5 minutes for staining. The stained sections were mounted in glycerin-gelatin to obtain permanent preparations (Jensen, 1962). Sections were examined using an Olympus light microscope BX51. Images were taken with an Olympus DP 70.

For palynological investigations pollen grains supplied from herbarium specimens and were prepared following (Wodehouse, 1935). For measurements of polar axis, equatorial diameter, exine thickness and lumina size of pollen grains, 100x objective of a Olympus light microscope CX21 were used. Measurements were based on 20 pollen grains. For morphological descriptions of pollen grains, the terminology of Punt *et al.* (1994) was followed. Statistical studies were performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Morphological properties

C. astrantiae Boiss. & Bal. in Boiss., Fl. Or. 2: 906 (1872) (Hedge and Lamond, 1972) (Figures 1a-9a).

Pilose to ± glabrous perennial with terete stems, branched or not, 30-60 cm. Lower leaves broad ovate-triangular, 10-20 × 7-8 cm, 2-3 pinnate; ultimate segments ovate, deeply pinnatisect with ± linear lanceolate lobes 2-6 × 0.5-1.0 mm. Rays 6-11, ± equal, 1-3 cm, erect. Bracts 0-1. Bracteoles 4-6, conspicuous, white, ciliate, broad elliptic to obovate, 5-9 × 3-4 mm. Pedicels shorter than bracteoles. Fertile flowers 4-10 per umbellule. Petals ± equal. Fruit oblong, 5-8 × c. 1.5 mm. *Fl.* 8. *Rocky places, 2130-3200 m.*

C. aureum L., Sp. Pl. ed. 2:370 (1762) (Hedge and Lamond, 1972) (Figures 1b-9b).

Almost glabrous to shortly pilose perennial with branched terete or angled, often spotted stems to c. 90 cm. Lower leaves broad-ovate to triangular, 2-3-pinnate, 15-30 × 9-20 cm, ultimate segments ovate, elliptic or broadly lanceolate, acutely lobed, lobes crenate or sharply serrate. Rays 15-25, unequal, 1.5-5 cm, ± erect in fruit. Bracts 0-1. Bracteoles 5-7, lanceolate, ciliate, 3-6 × 1 mm. Pedicels shorter than fruit. Fertile flowers 5-8 (-12) per umbellule. Fruit oblong to ovate-oblong, 7-10 × c. 3 mm, pale brown when mature. *Fl.* 6-8. *Rocky slopes in Picea-Rhododendron scrub, open grassy meadows, 1000-2300 m.*

Anatomical properties

In root cross sections, roots have secondary structure. Periderm consists of three different tissues: phellem with depressed rectangular cells (3-7 layers in *C. astrantiae*, 5-7 layers in *C. aureum*), phellogen (1 layered in both species) and phelloderm (1 layered in both species), from exterior to interior.

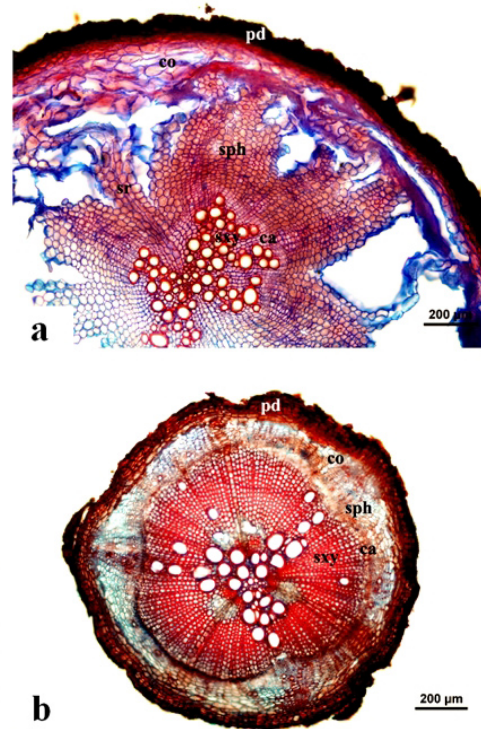


Fig. 1. Cross section of roots: a. *C. astrantiae*; b. *C. aureum* (ca: cambium; co: cortex; pd: peridermis; sph: seconder phloem; sxy: seconder xylem) (Scale 200 μm)

Cortex consists of 3-6 layered parenchymatic tissue in *C. astrantiae* and 4-7 layered parenchymatic tissue in *C. aureum*. Parenchymatic tissues have thickness of 125-288 μm in *C. astrantiae* and 50-115 μm in *C. aureum*. Cambium consists of 2-3 layers in *C. astrantiae* and 3-4 layers in *C. aureum*. In cortex, there are secretory ducts surrounded by 4-8 glandular cells in both of the species (Figs. 1-2).

When cross sections of stems were examined, it is determined that thickness of cuticle were found to be 5-8 μm in *C. astrantiae* and 3-5 μm in *C. aureum*. The epidermis consists of ovoidal cells with 15-24 × 6-10 μm in size in *C. astrantiae* and 12-30 × 10-18 μm in size in *C. aureum*. Under epidermis, parenchymatous cortex is 7-9 layered in *C. astrantiae* while 11-14 layered in *C. aureum*. Cells of cortex are 15-38 × 7-20 μm in size in *C. astrantiae* while 10-55 × 7-45 μm in size in *C. aureum* and are ovoidal in shape. In cortex, it is determined that collenchyma cells have 6-9 layers in *C. astrantiae* and 10-13 layers in *C. aureum*. There are secretory ducts surrounded by 4-8 glandular cells in *C. astrantiae* and 6-13 glandular cells in *C. aureum*.

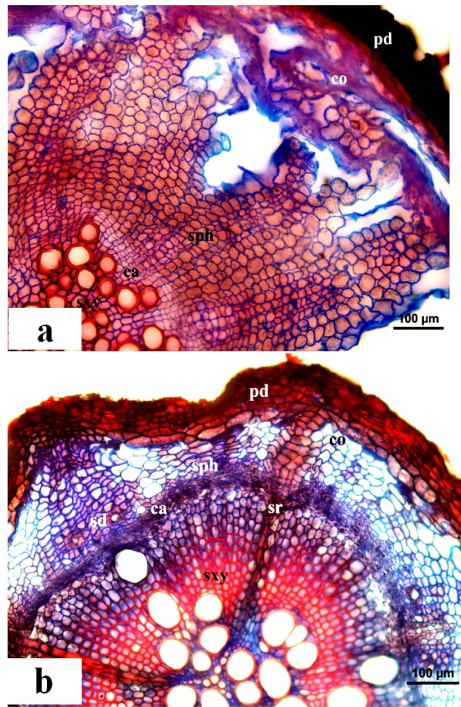


Fig. 2. Cross section of roots in detail: a. *C. astrantiae*; b. *C. aureum* (co: cortex ca: cambium; pd: peridermis; sph: seconder phloem; sr: seconder pith ray; sxy: seconder xylem;) (Scale 100 µm)

Center of the stem (pith region) is made of circular to circular-ovoid parenchymatic cells which have the sizes of 12-93 × 10-82 µm in *C. astrantiae* and 48-125 × 37-105 µm in *C. aureum* (Figs. 3-4).

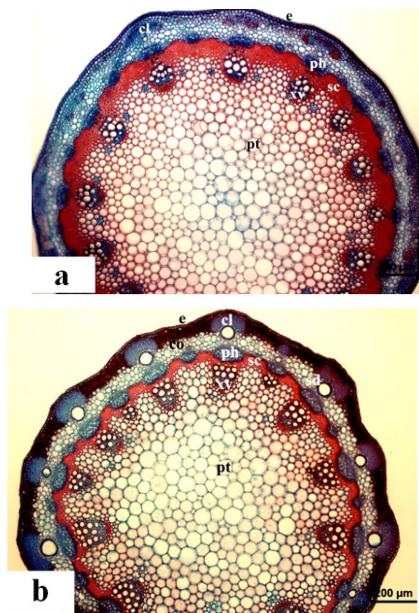


Fig. 3. Cross section of stems: a. *C. astrantiae*; b. *C. aureum* (cl: collenchyma; co: cortex; e: epidermis; ph: phloem; pt: pith; sc: sclerenchyma; sd: secretory duct; xy: xylem; xys: sclerenchyma of xylem) (Scale 200 µm)

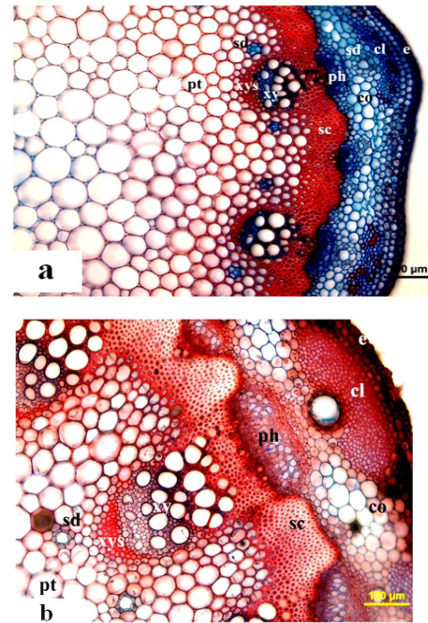


Fig. 4. Cross section of stems in detail: a. *C. astrantiae*; b. *C. aureum* (cl: collenchyma; co: cortex; e: epidermis; ph: phloem; pt: pith; sc: sclerenchyma; sd: secretory duct; xy: xylem xys: sclerenchyma of xylem) (Scale 100 µm)

In stem leaf anatomy, it was observed that the shape of epidermal cells are oblong-ovoidal in both of the species. There are unicellular hairs on leaf surface rarely in *C. aureum*. Upper epidermis cells are 15-25 × 6-12 µm in size in *C. astrantiae* and 12-55 × 7-30 µm in *C. aureum*. The leaves are amphistomatic and mesophyll is bifacial in both species (Fig. 5).

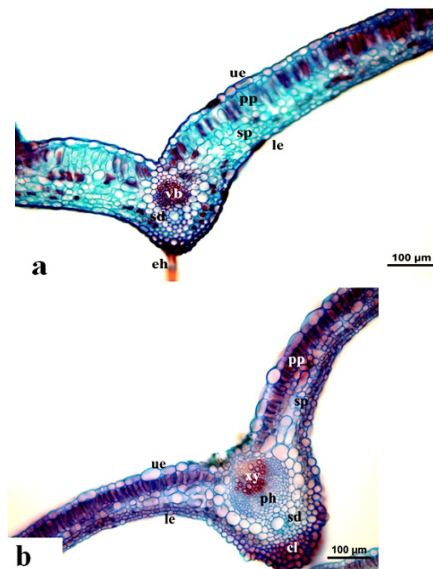
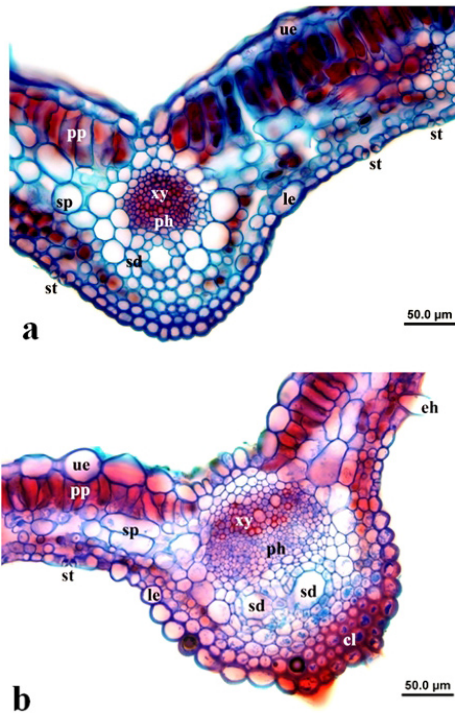


Fig. 5. Cross sections of stem leaves: a. *C. astrantiae*; b. *C. aureum* (cl: collenchyma; eh: eglandular hair; le: lower epidermis; ph: phloem; pp: palisade parenchyma; sd: secretory duct; sp: spongy parenchyma; ue: upper epidermis; vb: vascular bundle; xy: xylem) (Scale 100 µm)

Palisade parenchyma cells generally cylindrical in shape are 1 layered and have the sizes of $35-55 \times 7-13 \mu\text{m}$ in *C. astrantiae* and $17-38 \times 7-15$ in *C. aureum*. Spongy parenchyma cells are 4-6 layered and $10-38 \times 7-23 \mu\text{m}$ in size in *C. astrantiae* while 3-4 layered and $10-50 \times 7-27 \mu\text{m}$ in *C. aureum*. They are circular, ovoid or rarely irregular shaped. There are secretory ducts consists of 9-13 cells in areas corresponding to the main vascular bundle between lower epidermis and vascular bundle in both species (Figs. 5-6).

Fig. 6. Cross sections of stem leaves in detail: a. *C. astrantiae*;



b. *C. aureum* (cl: collenchyma; eh:eglandular hair; le: lower epidermis; ph: phloem; pp: palisade parenchyma; sd: secretory duct; sp: spongy parenchyma; st: stoma; ue: upper epidermis; vb: vascular bundle; xy: xylem) (Scale 50 μm)

Fruits are schizocarpic which made of two mericarp in both of the examined species. (Fig. 7).

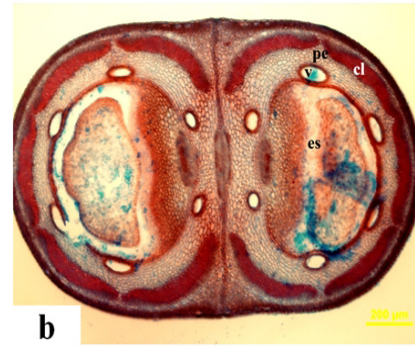


Fig. 7. Cross sections of fruit: a. *C. astrantiae*; b. *C. aureum* (cl: collenchyma; es: endosperm; pe: pericarp; sd: secretory duct; v: vittae) (Scale 200 μm)

Exocarp layer have oblong-ovoidal cells with $15-40 \times 7-13 \mu\text{m}$ in size in *C. astrantiae* and $7-25 \times 5-10 \mu\text{m}$ in *C. aureum*. Above the exocarp cuticula is $3-5 \mu\text{m}$ in size in both species. Below the exocarp there is mesocarp layer which consists of oblong-ovoidal, pentagonal or hexagonal cells. Mesocarp is 10-14 layered in *C. astrantiae* while 17-23 layered in *C. aureum*.

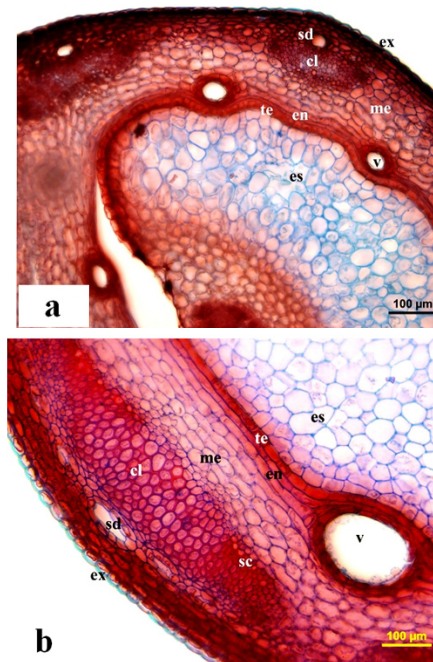


Fig. 8. Cross sections of fruit mericarp in detail: a. *C. astrantiae*; b. *C. aureum* (cl: collenchyma; en: endocarp; es: endosperm; ex: exocarp; me: mesocarp; sc: sclerenchyma; sd: secretory duct; te: testa) (Scale 100 μm)

In the middle of the mesocarp layer there are 7-9 layered collenchyma in *C. astrantiae* and 4-9 layered collenchyma in *C. aureum*. Inner layer of pericarp that endocarp is single layered in both species and endocarp consists of very pressed rectangular cells which have the sizes of $37-90 \times 5-10 \mu\text{m}$ in *C. astrantiae* and $45-74 \times 6-9 \mu\text{m}$ in *C. au-*

reum. Between the mesocarp and endocarp layer 2 vittae (oil duct) are seen on the commissural face while 4 vittae are seen on lateral and dorsal faces in both of the species. Diameter of vittae opening in *C. aureum* is larger than *C. astantiae*. Testa is single layered in both species and cells are $12-43 \times 12-18 \mu\text{m}$ in size in *C. astantiae* while $15-50 \times 5-13 \mu\text{m}$ in *C. aureum* (Figs. 7-8).

Palynological properties

In palynological study, *C. astantiae* and *C. aureum* have tricolporate pollen type, perprolate pollen shape and perforate exine ornamentation. Under the pore there is a costa nearly $1.01 \mu\text{m}$ in pollen grains of both species. The ratio of P/E is approximately 2.10 making the pollen grain shape perprolate (Erdtman, 1952) in both species. Exine surface ornamentation is coarsely perforate under light microscope. (Fig. 9, Tab. 1).

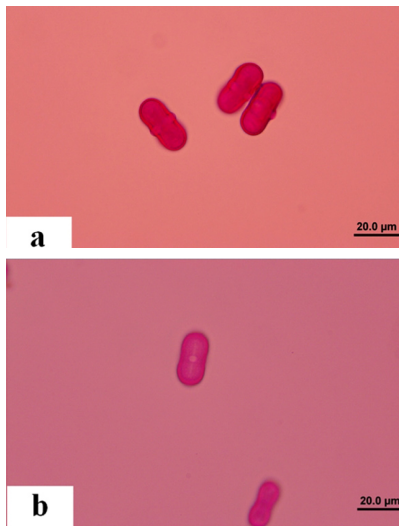


Fig. 9. Morphology of pollen grains under light microscope: a. *C. astantiae*; b. *C. aureum* (Scale: 20.0 μm)

Discussion

There are many studies about chemical properties of the genus *Chaerophyllum* L., mainly on the composition of essential oils in *C. macrospermum* (Spreng.) Fisch CA Mey, *C. aromaticum*, *C. macropodum* Boiss, *C. critinum* Boiss, *C. aksekiense* (Razavi and Nejad-Ebrahimi, 2010; Chizzola, 2009; Nematollahi *et al.*, 2005; Baser *et al.*, 2000), antimicrobial activity of essential oils in *C. aureum* (Lakusić *et al.*, 2009), antioxidant compounds of *C. hirsutum* (Dall'Acqua and Innocenti, 2004) and flavonoid glycosides in *C. aureum* (Gonnet, 1985). In recent years, there were lots of studies about Apiaceae. Including morphology, anatomy and palynology of some genus in the family. In 2005, Applequist examined the root anatomy of *Ligusticum* species (Apiaceae) which was sold as Osha. The herbal medicine Osha consisted of the roots of *Ligusticum porteri* J. M. Coult. & Rose (Apiaceae). In that study,

the anatomy of taproots and rootstocks of several species of *Ligusticum* was described and illustrated (Applequist, 2005). The wood anatomy of *Bupleurum* L. (Apiaceae, Apiaceae) in relation to habit, phylogenetic relationships, and infrageneric taxonomy was studied (Stepanova and Oskolski, 2010). Morphological, carpo-anatomical characters, pollen characteristics, mericarp surface features of *Bilacunaria anatolica* sp. nov. (Apiaceae) from southwest Anatolia in Turkey were studied (Duran *et al.*, 2012). In recent years, the new monotypic genus *Siculosciadium* (Apiaceae) from Sicily was determined (Brullo *et al.*, 2013). Uruşak and Kızılarıslan (2013) studied the comparative fruit anatomical properties based on mericarp shape in transverse sections of 27 *Ferulago* species (Apiaceae) grown in Turkey. The studies on anatomical and palynological studies on *Chaerophyllum* taxa both in the world and in Turkey are not much in number. *Chaerophyllum* species including antioxidant compounds and active in terms of microbial activity are pharmacologically important taxa. Palynological and anatomical characteristics of the species rich in essential oils should be defined clearly to make comparative studies between taxa. The present study is therefore important as it provides comparative data to be used in studies within the genus.

Tab. 1. Palynological properties of *C. astantiae* and *C. aureum* (P: polar axis, E: equatorial axis, Clg: colpus length, Clt: colpus width, Plg: pore length, Plt: pore width)

Pollen features	Mean \pm Standard Deviation (Min-Max) (μm)	
	<i>C. astantiae</i>	<i>C. aureum</i>
P	25.87 \pm 0.69 (25.37–27.40)	23.95 \pm 0.76 (22.33–25.37)
E	12.02 \pm 0.37 (11.16–12.18)	11.67 \pm 0.52 (11.16–12.18)
P/E ratio	2.15	2.05
Pollen shape	Perprolate	Perprolate
Pollen type	Tricolporate	Tricolporate
Clg	13.09 \pm 0.92 (11.16–15.22)	12.07 \pm 1.08 (10.15–13.19)
Clt	1.72 \pm 0.25 (1.52–2.03)	1.64 \pm 0.40 (1.01–2.03)
Plg	3.49 \pm 0.32 (3.04–4.06)	3.29 \pm 0.26 (3.04–3.55)
Plt	5.47 \pm 0.51 (5.07–6.09)	5.24 \pm 0.24 (5.07–5.58)
Exine thickness in equatorial axis	1.01 \pm 0.00 (1.01–1.01)	1.52 \pm 0.00 (1.52–1.52)
Exine thickness in polar axis	1.01 \pm 0.00 (1.01–1.01)	1.77 \pm 0.26 (1.52–2.03)
Ornamentation type	coarsely perforate	coarsely perforate
Costa	1.01 \pm 0.00 (1.01–1.01)	1.01 \pm 0.00 (1.01–1.01)

Conclusion

This study is important for the taxonomy of *Chaerophyllum* genus. This is the first report on the anatomy and detailed palynology of *C. aureum* and *C. astrantiae*. In this study, root, stem, stem leaf and fruit anatomy and palynological characters of *C. astrantiae* and *C. aureum* were investigated in detail for the first time.

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