

A new hybrid record of the genus *Lophiolepis* (= *Cirsium* sect. *Epitrachys*) (Asteraceae, Carduinae) from Türkiye

Lophiolepis (= *Cirsium* *Epitrachys* seksiyonu) cinsine ait Türkiye’den yeni bir melez kaydı (Asteraceae, Carduinae)

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Abstract

Lophiolepis Cass., belonging to the tribe Cardueae of the family Asteraceae (subtribe Carduinae) is newly separated from the genus *Cirsium* Miller in the light of recent molecular studies. It has a high affinity to form interspecific and intersubspecific hybrids under certain circumstances. In the present study, one hybrid combination, *Lophiolepis x trifurca* (Petr.) Bureš, Del Guacchio, Iamonico & P. Caputo which was previously described as *Cirsium x trifurcum* Petrak by Petrak in 1914 is newly recorded for Turkish Flora. The hybrid specimens have intermediate characters with parental species; *Lophiolepis kosmelii* (Adams) Bureš, Del Guacchio, Iamonico & P. Caputo and *L. caucasica* (Adams) Bureš, Del Guacchio, Iamonico & P. Caputo. Plant samples including parental species were collected from Şavşat district of Artvin province in sympatric populations. These specimens were carefully examined and compared with the putative parents, and morphological character measurements of hybrid plants, as well as parental species were carried out. The samples' natural habits were photographed, cauline leaf drawings were supplied using a stereomicroscope, leaf micromorphological characters were determined and anatomical characteristics were compared with parents, in detail. In conclusion, intermediate morphological characteristics between *L. x trifurca* and its parents were determined in terms of features such as cauline leaves, phyllaries, capitula and corolla. It has spines on adaxial sides, and uniseriate multicellular tomentose trichomes in abaxial ones. Cuticle has striate appearance near to the stomata/ trichome bases in the abaxial surface and scale/granules are shown in adaxial parts. This taxon has amphistomatic leaves and dorsiventral mesophyll as in the parental species.

Özet

Lophiolepis Cass., Asteraceae familyasının Cardueae tribusu (alttribus Carduinae)’nda yer alan ve son yapılan moleküler çalışmaların ışığı altında *Cirsium* Miller cinsinden ayrılan bir cinstir. Cins, belirli koşullar altında türler ve alt türler arasında yüksek oranda hibrit oluşturma potansiyeline sahiptir. Bu çalışmada, önceleri Petrak tarafından 1914 yılında *Cirsium x trifurcum* Petrak olarak tanımlanan, *Lophiolepis x trifurca* (Petr.) Bureš, Del Guacchio, Iamonico & P. Caputo adlı bir melez Türkiye Florası için ilk defa rapor edilmektedir. Hibrit örnekler atasal taksonlar olan; *Lophiolepis kosmelii* (Adams) Bureš, Del Guacchio, Iamonico & P. Caputo ve *L. caucasica* (Adams) Bureš, Del Guacchio, Iamonico & P. Caputo ile araform özellikler sergilemektedir. Bitki örnekleri Artvin ili Şavşat ilçesinden atasal taksonların da mevcut olduğu simpatrik popülasyonlardan toplanmıştır. Bu örnekler dikkatlice incelenmiş ve muhtemel atasal taksonlar ile karşılaştırılmış olup atasal taksonlarla beraber hibrit bitkilerin morfolojik özelliklerinin ölçümleri gerçekleştirilmiştir. Örneklerin doğal görünüşleri fotoğraflanmış, gövde yaprakları stereomikroskop kullanılarak çizilmiş olup yaprak mikromorfolojik özellikleri tanımlanmış ve anatomik özellikleri atasal taksonlar ile detaylı olarak karşılaştırılmıştır. Sonuç olarak, *L. x trifurca* ile atasal taksonlar arasında gövde yaprakları, fillariler, kapitulum ve korolla açısından araform morfolojik özellikler tespit edilmiştir. Takson, yaprak üst yüzeylerinde sık dikenler, alt yüzeylerinde ise tek sıra çok hücreli keçe tüyler içermektedir. Kütikula, alt yüzeylerde stoma/tüy tabanı yakınlarında çizgili görünümünde olup, üst yüzeylerde ise pul/granüller görülmektedir. Bu taksonun yaprakları atasal taksonlarda olduğu gibi amfistomatik olup dorsiventral mezofillidir.

INTRODUCTION

The genus *Cirsium* Mill., known as thistles, is one of the largest genera in Asteraceae (Cardueae). It is composed of ca. 250-300 species (Davis and Parris 1975, Petrak 1979, Charadze 1998, Zhu and Werner 2011), 400-450 species (Bures et al. 2018) or 459 taxa (POWO 2023)

depending on the authors in the world. They are mainly distributed in Europe, North Africa, East Asia, Central Asia, SW Asia, and North and Central America from subtropical to boreal latitudes (Davis and Parris 1975, Charadze 1998, Bures et al. 2018). Recently, several new species were added to the Turkish Flora (Yıldız et al. 2012,

Yıldız et al. 2016, Duman et al. 2017). According to recent reports, *Cirsium* is represented by 68 species (80 taxa, 33 endemic) including the *C.* sect. *Epitrachys* (50 species, 52 taxa), *C.* sect. *Cirsium* (17 species, 27 taxa), and *C.* sect. *Cephalonoplos* (Neck.) DC. (1 species) in Türkiye (Davis and Parris 1975, Yıldız et al. 2012, Yıldız et al. 2016, Duman et al. 2017).

The species of the genus, especially belonging to sect. *Cirsium*, have a strong affinity to produce interspecific hybrids in nature. Till now, several hybrid taxa have been recorded from different countries (i.e. 16 taxa from Russia etc.) (Wagenitz 1987, Bobrov and Cherepanov 1998, Bureš et al. 2004, 2010, Segarra-Moragues et al. 2007, Bures et al. 2018, Michálová et al. 2018, Sheidai et al. 2018), as well as from Türkiye (Yıldız et al. 2016, Dirmenci et al. 2019, Yıldız et al. 2019). Three new hybrids belong to *C.* sect. *Epitrachys* (Dirmenci et al. 2019) and two new hybrid records from the *C.* sect. *Cirsium* (Yıldız et al. 2016) had been determined in recent reports. In addition, one hybrid taxon has been newly recorded from Konya (Türkiye) (Yıldız et al. 2019).

It has been known that the genus *Cirsium* is paraphyletic (Häffner 2000, Barres et al. 2013, Herrando-Moraira et al. 2019), and because of general resemblance among species within *Cirsium* and related genera, generic delimitations and infrageneric taxonomy are rather complicated and controversial (Del Guacchio et al. 2022). Based on morphological characters, this genus is classified into several sections (Davis and Parris 1975, Werner 1976, Petrak 1979, Charadze 1998); one of them, sect. *Epitrachys* DC. (= sect. *Eriolepis* (Cass.) Dumort. contains more than 100 species. Member of this section has large capitula and persistent stiff spines on the upper surface of leaves (Davis and Parris 1975). In light of recent molecular studies, Del Guacchio et al. (2022) proposed the *Cirsium* sect. *Epitrachys* DC. (sect. *Eriolepis* (Cass.) Dumort.) as a separate genus; *Lophiolepis* Cass. excluding *C. italicum*.

During fieldwork studies, some interesting hybrid samples between *Lophiolepis kosmelii* and *L. caucasica*, previously known as *Cirsium kosmelii* and *C. caucasicum*, were collected between 2008 and 2017. As well as Turkish

Flora (Davis and Parris 1975, Davis et al. 1988) and adjacent floras; Flora Severnogo kavkaza (Galushko 1978-1980), Flora Romaniei (Grecescu 1898), Flora USSR (Bobrov and Cherepanov 1998), Flora of China (Zhu and Werner 2011), Flora Iranica (Petrak 1979) had been checked. *L. x trifurca* (as *C. x trifurcum*) was previously described as a new hybrid by Petrak in 1914 from Bakuriani (Georgia). It is naturally distributed North Caucasus (POWO 2023).

The aim of the present study is to describe the new *Lophiolepis* (= *Cirsium* sect. *Epitrachys* DC.) hybrid record for the flora of Türkiye, to determine the morphological differences between the hybrid and its parental species and to compare micromorphological and anatomical data among them. Differences of the new hybrid record from its parental species (*Lophiolepis kosmelii* and *L. caucasica*) are presented in Tables 1 and 2, in detail.

MATERIAL AND METHODS

Plant Samples

Examined samples were collected from natural populations in Artvin, Türkiye. Specimens for morphological examinations were dried according to standard herbarium techniques and stored at Artvin Coruh University Herbarium (ARTH). Parental species were identified according to Turkish Flora (Davis and Parris 1975) and *L. x trifurca* was checked from the plant image obtained in the physical collections of Moscov University Herbarium (MW) (Seregin 2023).

Micromorphological Analysis

Small and dry parts of leaf midrib, lamina and margins from upper and lower surfaces were separately placed on stubs and observed using SEM microscope at an acceleration voltage of 10 kV (Ozcan and Demir 2022). All parts including the midrib, lamina, and margins both of the upper and lower surfaces of the taxon were examined and micrographs were taken. The terminology of the leaf characters proposed by Barthlott (1981) and Stearn (1985) is used to describe cell arrangements and surface ornamentation.

Anatomical Preparations

Fresh cauline leaves were fixed in the field with formalin–acetic acid–alcohol (FAA), or removed from herbarium material. Cross sections were prepared from the median part of the laminae. Transverse and paradermal sections of leaves were obtained by hand with the aid of razor blades. Double staining was performed for cross sections with safranin–alcian blue and haematoxylin solution was used for paradermal sections. Excess staining was removed from the sections by washing them in water several times (Algan 1981, Ozcan and Yilmaz 2020). Semi-permanent slides were mounted in glycerin solution (Vardar 1987). Slides were examined under light microscopy and well-stained sections were examined under light microscopy and photographed using an Olympus BX53 research microscope with digital camera attachment DP73.

To evaluate anatomical characters, several cross-sections were obtained from three to five plant samples and calculated the means and standard error among different cross-sections. Ten paradermal slides (five from the upper and five from the lower surfaces) were evaluated and 50 stomatal lengths were measured on each surface. Epidermal and leaf structural features were described according to the terminology of Metcalfe and Chalk (1979) and the stomatal index was calculated according

to the method described by Meidner and Mansfield (1968).

RESULTS AND DISCUSSION

Lophiolepis × *trifurca* (Petr.) Bureš, Del Guacchio, Iamónico & P. Caputo (2022). In: Pl. Biosystems 156(5): 1286; *Cirsium* × *trifurcum* Petrak in Izv. Kavk. Muz. VIII, 1-2 (1914) 59. Exs.: Petrak, Cirsioth, Univ. No. 140 (Bobrov and Cherepanov 1998).

(Fig. 1).

Type: Georgia, Bakuriani

Examined Specimens: Türkiye: Artvin, Şavşat, Ardahan road, 2329 m, 41°12'40.4"N, 42°28'08.1"E, 31 Aug 2008, M. Ozcan 237; Artvin, near Ardahan Çam pass, 2411 m, 29 Jun 2017, 41°12'06.9"N, 42°31'19.3"E, M. Ozcan 829. Scan of the image of *L. x trifurca* (as *C. x trifurcum*) from Moscow University Herbarium (MW).

Distribution: North Caucasus. New to Türkiye (Artvin).

Ecology: This hybrid occurs in roadsides at 2329–2411 m altitudes where parental species naturally grow in. They share sympatric habitats with their parental species; *L. kosmelii* and *L. caucasica*.

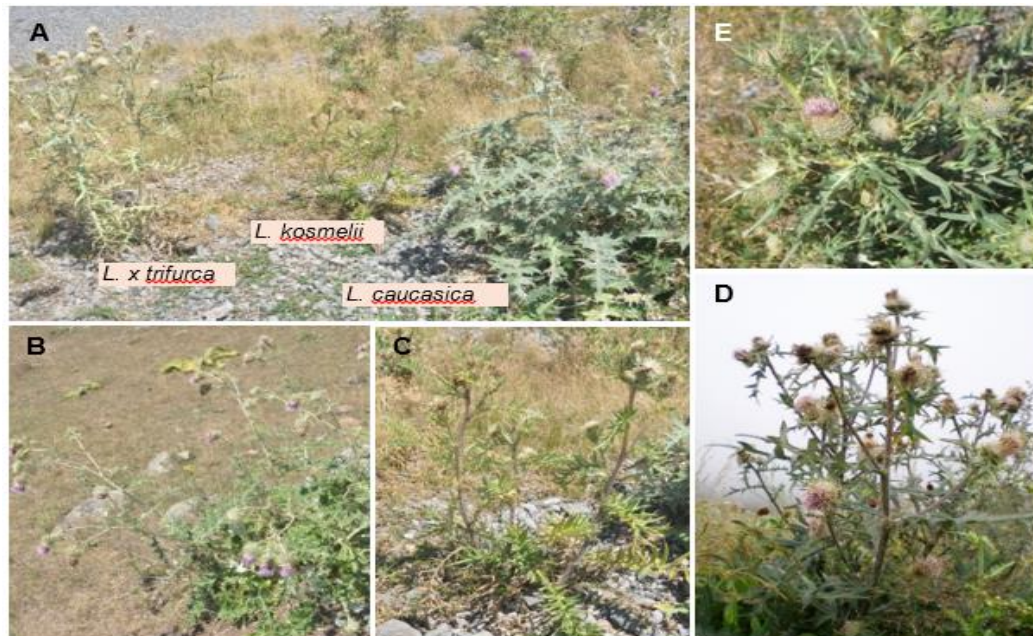


Figure 1. Natural habitus of *Lophiolepis x trifurca* with parental species.

A: three taxa found in same population, B: *L. caucasica*, C: *L. kosmelii*, D: *L. x trifurca*, E: young hybrid plant

Petrak reports that this hybrid is very rare among species of the section *Epitrachys* DC. and almost 70% of the pollen is not fully viable. The parental species; *L. kosmelii* (= *C. kosmelii*) is widespread in Southern Transcaucasia, whereas the southern boundary of *L. caucasica* (= *C. caasicum*) runs through the Trialeti Range, where hybrids are observed (Bobrov and Cherepanov 1998).

Morphological Comparison of the Hybrid with the Parental Species

Lophiolepis x trifurca is distinguished from *L. kosmelii* by its much-branched stem in the upper part (vs. single or few branches) and sometimes purple appearance, leaves broadly pinnate lobes (vs. divided to the midrib), upper stem leaves pinnatifid to pinnatipartite (vs. deeply

pinnatisect), involucre bracts mostly 2-3, more or less equal to capitula (no longer), with tomentose indumentum (vs. arachnoid to sparsely trichomes), leaf lobes 5–7 pairs (vs. 6–8 pairs), corolla pale pinkish-lilac to dirty white (vs. yellowish to sordid white). It can be distinguished from *L. caucasica* by erect involucre (vs. nodding), 6-7 seriate (vs. 11-12 seriate), purple, erect (vs. white and spreading), sparsely arachnoid stem with many long cauline leaves up to the tips (vs. diminished from base to inflorescence), densely short branched in upper part (vs. few and long branches), leaf lobes 5–7 pairs (vs. 6–8 pairs), and apical spine of median phyllaries 5-7 mm (vs. 6-10 mm), corolla pale pinkish-lilac to dirty white (vs. pinkish to purple) (Figs. 2 and 3). Detailed morphological characters are presented in Table 1.

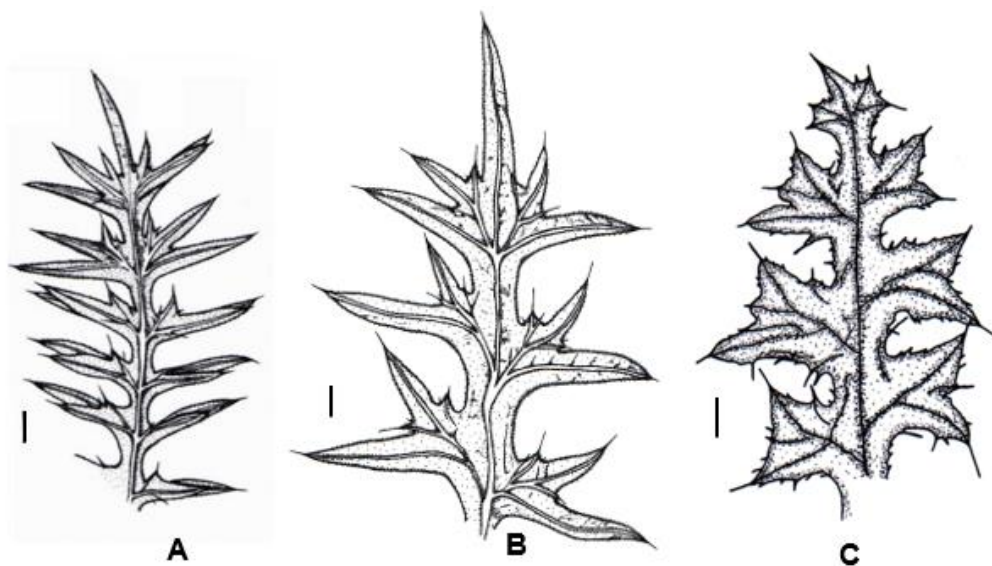


Figure 2. Cauline leaves. A: *Lophiolepis kosmelii*, B: *L. x trifurca*, C: *L. caucasica*. Scale bar: 1 cm

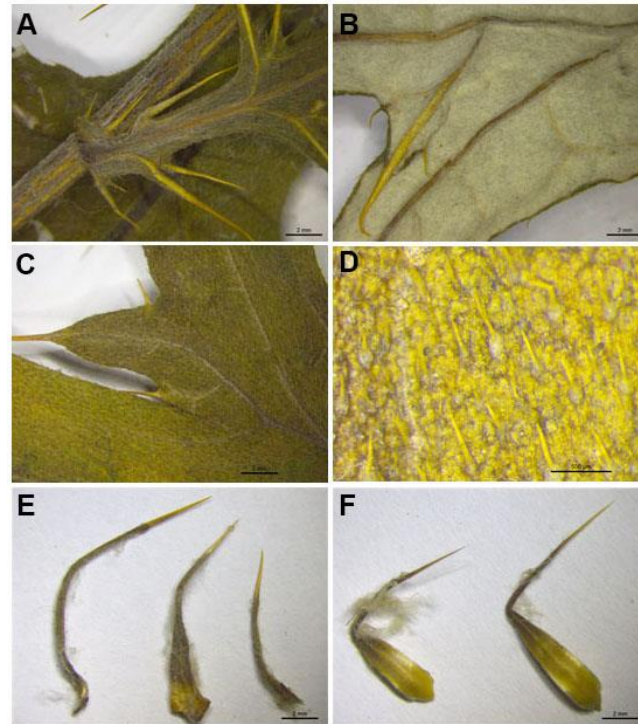


Figure 3. Leaf and phyllaries of *Lophiolepis x trifurca*. a: leaf base, b: lower surface, c: upper surface, d: spine/spinules in the upper surface, e: outer phyllaries, f: median phyllaries. Scale bars: 2 mm (a, b, c, e, f), 500 µm (d)

Table 1. Comparison of morphological characters of *Lophiolepis x trifurca* taxa with parental species

Character		<i>L. x trifurca</i>	<i>L. kosmellii</i>	<i>L. caucasica</i>
Stem	Colour/indumentum	Whitish to purple with long sparsely trichomes	Purplish with densely arachnoid trichomes	Whitish, long arachnoid thichomes
	Length/	80-90 cm	30-55 cm	45-100 cm
	Branching	3-4 long branches	0-7 short branches	3-4 short branches
Involucre	Position	erect	erect	nodding
	Size	21-24 x 23-27 mm	22-28 x 19-25 mm	23-30 x 20-45 mm
	Indumentum	tomentose	arachnoid to sparsely trichomes	tomentose
Capitula	Size	1-2 capitula, 21-24 mm x 23-27 mm	1 capitula, 22-28 x 19-25 mm	1-2 capitula, 23-30 x 20-45 mm
	Shape	Widely obovoid	Cylindrical to obovoid	Globose-obovoid
Involucral bracts	Outer phyllary	Densely arachnoid, 14-18 mm x 1.75-2.52 mm with 3.12-3.48 mm terminal spine	Arachnoid, 9.41-10.25 x 2 mm with 2.33-2.91 mm terminal spine	Densely arachnoid, 26.5 x 2.83 mm with 2.5 mm terminal spine
	Median phyllary	More or less erect, spines with different sizes, 14-16 x 2 mm (terminal spine length 5.88-6.6 mm)	Erect, 13.66-14,50 x 2.08 mm (terminal spine length 2.41-2.83 mm)	Nodding, 29-31 x 2,5-3 mm (terminal spine length 6-7 mm)
	Number of series	6-8 seriate	7 seriate	11-12 seriate
Cauline leaves	Shape	Oblong, deeply pinnatifid to pinnatipartite, lateral lobes bifid, more or less divided to the midrib	Oblong, pinnatisect, 4-6 paired bifid, widely divided	Oblong, pinnatifid to pinnatipartite, 4-6 paired bifid, divided up to 2/4
	Spine length in adaxial surface	0.25-0.55 (up to 1.20 mm)	0.25-0.80 mm	0.25-0.5 mm (up to 3 mm)
	Spine length of terminal/lateral segment	5-7 mm, moderately stout	3.40-4.5 mm, weak	6-10 mm, stout
Flower colour		Pinkish-lilac to sordid white	Yellowish, sordid white	Pinkish to purple
Pappus		Light reddish brown or sordid-white, 18-20 mm	Yellowish-sordid white, 17-21 mm	Yellowish –white, 24-27 mm

Micromorphological Results

Leaves have spines and spinules in the adaxial sides and margins because of belonging to the sect. *Epitrachys*, and woolly indumentum in the abaxial ones, as in the parental species. In addition, simple uniseriate multicellular trichomes with different sizes and basal diameters are separately distributed on the upper surface, while they have long wipe-like tips seen sparsely on adaxial surface and with large numbers, showing densely tomentose indumentum on the abaxial sides. Epidermal cells are long polygonal and straight and concave anticlinal cell walls and convex periclinal ones were shown in adaxial sides and cuticle ornamentation with small scales/small granules.

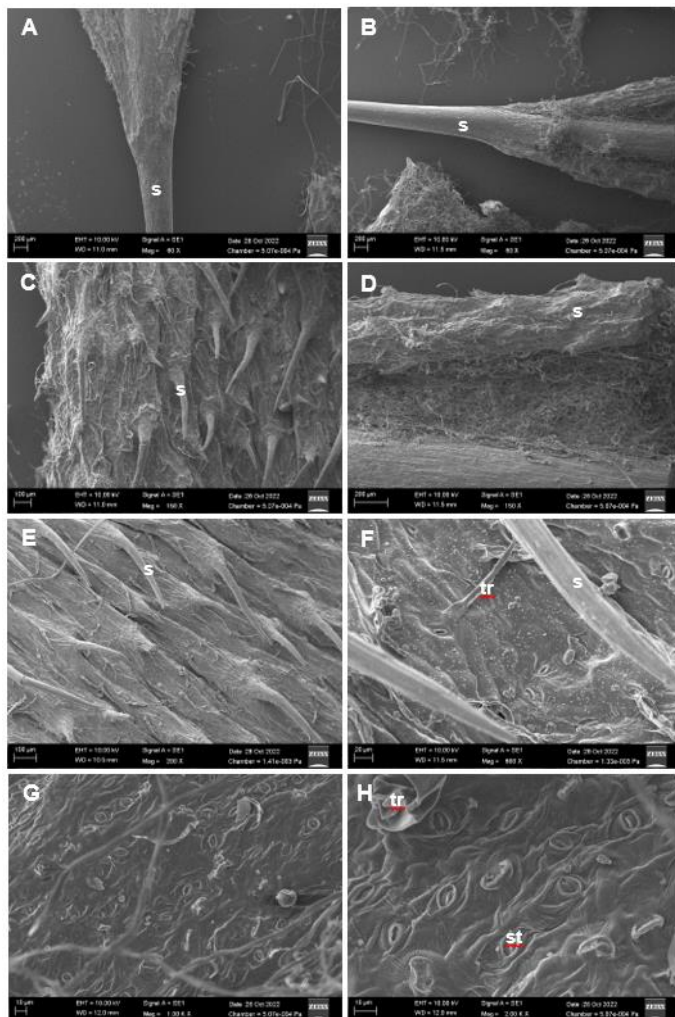


Figure 4. Leaf SEM micrographs of *L. x trifurca*. A, B: leaf tip, C, D: leaf margin, E, F: adaxial surface, G, H: abaxial surface.
s: spine, st: stoma, tr: simple trichome

They are more or less sinuous and concave anticlinal cell walls in abaxial parts and cuticle has a striate appearance among the stomata and near the bases of trichomes (Figs. 4 and 5).

Anatomical Results

The main leaf anatomical features observed through light microscopy are summarized in Table 2 and Fig. 5 comparatively to the parental species.

The adaxial epidermal cells of the taxon are polygonal with five to seven angles and straight cell walls in adaxial surfaces and slightly undulate to sinuous in abaxial ones. Similarly, anticlinal cell walls of parental *Lophiolepis* taxa are usually polygonal with usually straight cell walls. Whereas, the epidermal cell walls of *L. kosmelii* and *L. caucasica* were slightly undulate and sinuous on the abaxial surfaces, respectively. Stace (1965) noted that xeromorphic plants have more commonly straight epidermal cell walls, while mesomorphic plants typically undulate cell walls pattern. Gomes and Lombardi (2010) stated that the cuticle of shade leaves hardens more slowly and its walls remain frail and plastic for longer periods, thus favouring the development of sinuosity. In some leaves, the epidermal walls harden more quickly, thus tending to be less undulate. Fahn (1990) also pointed out that the epidermal cells of most leaves of shade-loving plants have sinuous anticlinal walls. Such sinuosity is probably due to the tensions that occur in the leaf and to cuticle hardening during cell differentiation (Alquini et al. 2003).

Simple trichomes consist of 1–2 small or 2–7 large basal cells and a long whip-like terminal cell (Figs. 5a₃ and b₃). These trichome types have previously been reported in the parental *Lophiolepis* (= *Cirsium*) species of this hybrid by Ozcan et al. (2015). Because of the taxa belong to the section *Epitrachys*, they have also spinules/ setae in the adaxial parts. Densely crowded simple trichomes are visible in the abaxial sides of all taxa, especially more distinctive in *L. caucasica*. Small glandular trichomes are also sparsely visible in the surfaces.

The taxon has amphistomatic leaves and stomatal density distinctly differs in adaxial and abaxial surfaces. Stomata

are anomocytic to anisocytic type and occur at the same level with the epidermal cells. These types of stomata have been previously reported in the family Compositae by Metcalfe and Chalk (1979) and Kadereit and Jeffrey (2007), and also in the genus *Cirsium* by Ozcan et al.

(2015). Mean stomatal densities are higher in the abaxial surfaces than the adaxial ones. The average stomatal frequencies were calculated as 124.0 ± 3.99 in adaxial parts and 404.0 ± 17.17 in abaxial parts of this hybrid. Mean stomatal size in adaxial and abaxial parts were

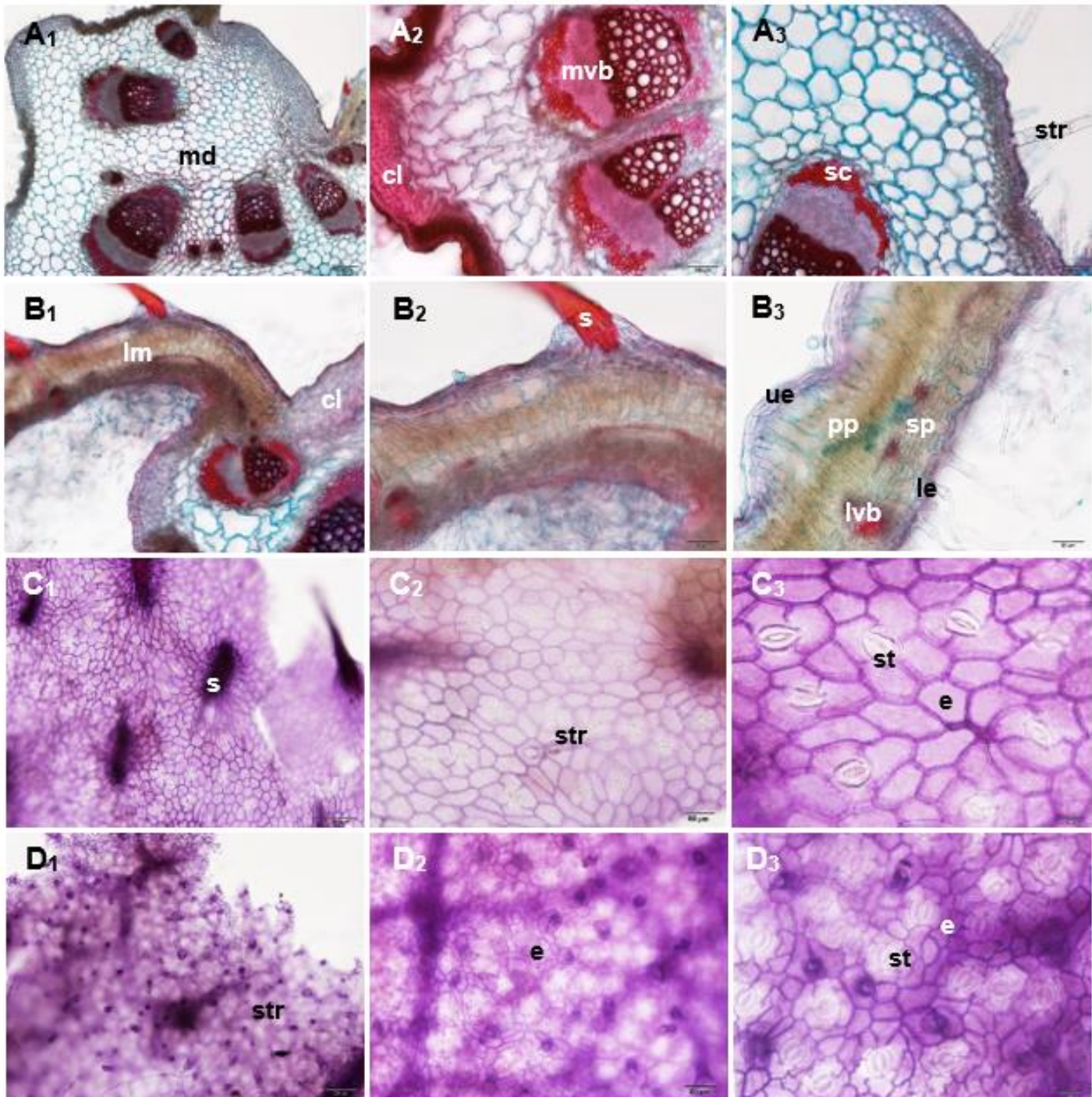


Figure 5. Leaf anatomical micrographs of *L. x trifurca*. a: midrib, b: lamina, c: adaxial epidermis, d: abaxial epidermis. cl: collenchyma, e: epidermis, le: lower epidermis, lm: lamina mesophyll, lvb: lateral vascular bundle, md: midrib, mvb: main vascular bundle, pp: palisade parenchyma, s: spine, sp: spongy parenchyma, st: stoma, str: simple trichome, ue: upper epidermis. Scale bars: 200 μ m (a1), 100 μ m (a2, a3, b1, c1, d1), 50 μ m (b2, b3, c2, d2), 20 μ m (c3, d3)

Table 2. Comparative leaf anatomical features of *L. x trifurca* with the parental species

Character		<i>L. x trifurca</i> (present study)	<i>L. kosmelii</i> (Ozcan et al. 2015)	<i>L. caucasica</i> (Ozcan et al. 2015)
Upper epidermis	length(μm)	24.74±3.06	19.40±2.33	33.33±1.67
	width (μm)	33.89±5.39	26.20±4.45	27.73±1.92
Lower epidermis	length(μm)	11.72±1.63	17.10±2.55	19.90±1.36
	width (μm)	17.79 ^a ±1.78	18.67±2.45	15.80±1.78
Number of collenchyma layers Upper/lower part of midrib		2-4/4-6	3-5/6-8	4-5/8-10
Midrib thickness (μm)		2432.56±287.9	3098.0±79.15	3996.0±213.34
Lamina mesophyll breadth (μm)		190.29±22.08	239.4±38.0	461.6±46.0
Palisade thickness (μm)		122.37±15.73	137.3±20.1	163.6±21.5
Spongy thickness (μm)		64.23±6.68	106.24±15.0	96.4±4.25
Row number of palisade/ spongy parenchyma		2-3/ 5-7	4-5	4-5
Trachea diameter (μm)		40.3±1.69	36.31±0.50	40.83±1.30
Xylem thickness (μm)		520.59±40.79	516.0±11.0	648.8±46.0
Phloem thickness		266.04±20.92	376.6±13.0	472.0±20.0
Central midrib Vascular bundle	Height (μm)	781.38±50.26	896.4±30.0	1116.0±49.0
	Width (μm)	525.39±22.91	457.6±12.0	676.8±31.0
Number of vascular bundle in midrib		5-6+ 3 (accessory bundle)	8-14 + 2-6 (accessory bundle)	6-17 + 3-9 (accessory bundle)
Adaxial surface	Cuticle thickness (μm)	3.59±0.33	2.92 ^a ±0.05	4.40±0.40
	Stomatal length (μm)	30.09±0.76	30.82±1.16	25.64±0.58
	Stomatal width (μm)	26.27±0.18	24.46±0.87	22.10±0.32
	Stomatal index	10.08±0.22	8.71±0.50	10.92±0.37
	Number of stomata (1 mm ²)	124.0±3.99	88.75±7.22	103.8±6.72
Abaxial surface	Cuticle thickness (μm)	3.07±0.18	2.10±0.10	2.20±0.37
	Stomatal length (μm)	22.23±0.19	23.54±0.38	18.80±0.65
	Stomatal width (μm)	19.92±0.43	21.20±0.07	17.14±0.62
	Stomatal index	12.77±0.29	14.45±0.42	13.75±1.03
	Number of stomata (1 mm ²)	404.0±17.17	385.0±18.68	720.0±78.68

*mean± standard error.

measured as 30.09±0.76 x 26.27±0.18 μm and 22.23±0.19x19.92±0.43 μm, respectively. Compared with the parental species (Ozcan et al. 2015), the hybrid has mean values in terms of stomatal characters (Table 2).

Leaves are bifacial (dorsiventral mesophyll) and are composed of a one-layered epidermis, palisade parenchyma, spongy parenchym and vascular tissue. Similar to parental species (Ozcan et al. 2015), it has two or three layers of compactly arranged palisade parenchyma, and five to eight layers of spongy parenchyma arranged with small spaces. Leaf lamina thickness of *L. kosmelii* and *L. caucasica* (as *C. kosmelii* and *C. causicum*) were reported as 239.4±38.0 and 461.6±46.0 μm. However, a thinner mean lamina mesophyll thickness was measured in this hybrid taxon as 190.29±22.08 μm. In previous reports, Metcalfe and Chalk (1979) and Kadereit and Jeffrey (2007), and Ozcan

et al. (2015) reported dorsiventral mesophyll for the Compositae family and for the genus *Cirsium*, respectively. These types of leaf mesophyll are also common in related genera, such as *Centaurea* L., *Psephellus* Cass., and *Silybum* Adans (Celik et al. 2008, Sibdu and Saini 2011, Aydin et al. 2013, Ozcan et al. 2014, Ozcan 2018, Yılmaz Çıtak et al. 2021). Midrib thickness was measured as 2432.56 μm. This value is thinner than both of the parental species (Ozcan et al. 2015). The adaxial surface of the midrib was reported as varying from flattish to concave in the species, but this is convex in the hybrid. 5-6 medium and three very small (accessory) bundles are located in midrib region. Accessory bundles have already been reported in many related taxa (Ozcan et al. 2014, Ozcan et al. 2015) and some authors proposed that these bundles are necessary to overcome unfavourable conditions during winter and meet the translocation requirements (Sidhu and Saini 2011).

Collateral vascular bundles are surrounded by parenchymatous sheath cells and some are very large and filled with secretory deposition. Large parenchymatic cells containing secretory material of various dimensions close to these bundles were previously reported by Ozcan et al. (2015). Similar to the parental species, *L. x trifurca*, has large parenchyma cells around the vascular bundles (Fig. 5a₂). Bundles are oblong in shape like those of *L. caucasica*, whereas *L. kosmelii* has widely oblong ones. One prominent and large vascular bundle and different numbers of accessory bundles were previously reported in the two parental species (Ozcan et al. 2015). Beneath the lower and upper epidermis of the lamina midrib, two to four and four to six layers of collenchyma are reported in the species as in this hybrid, respectively. More accessory bundles and collenchyma layers are found in the parental species than in this hybrid (Fig. 5, Table 2).

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