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COMPARATIVE ANATOMICAL STUDY OF LEAVES IN FIVE CUPRESSACEAE GRAY. TAXA IN KURDISTAN REGION - IRAQ

Ibrahim Z. Yousif [a,*], Saleem E. Shahbaz [a]
[a] College of Agricultural Engineering Sciences, University of Duhok, Duhok, Kurdistan Region, Iraq
(ibrahim.yousif; saleemshahbaz@uod.ac)

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ABSTRACT:

This study investigates the leaf anatomy of five Cupressaceae taxa in the Kurdistan Region-Iraq. Using the light microscope for numerical and descriptive anatomical variations of three species and two varieties belonging to *Cupressus*, *Platycladus*, and *Juniperus* genera were examined for Taxonomic value. These include the characters of epiderm, hypoderm, mesophyll layer, resin canals, transfusion tracheids, pith, and vascular bundles. There are significant differences among studied species and genera. *Cupressus* taxa exhibit a similar cross-sectional outline. *Cupressus arizonica* has a larger resin canal (125.4 µm) and longer palisade cells (91.8×27 µm) compared to *C. sempervirens* varieties. *Platycladus orientalis* form a wide rhombic outline, with an abaxial resin canal similar to *Juniperus oxycedrus* and *C. arizonica*. *J. oxycedrus* has a triangle outline with a thick hypoderm that extends below the entire epiderm, except under stomatal depressions. While in *Cupressus* and *Platycladus* taxa, the hypoderm is restricted only to the abaxial surface of the leaf. *J.oxycedrus* lacks pith entirely, unlike *Cupressus* and *Platycladus* taxa where they form polygonal or slightly straight pith at the center of the vascular bundle.

KEYWORDS: Cupressaceae, Cupressaceae anatomy, *Juniperus*, *Platycladus, Cupressus*.

1. INTRODUCTION

Cupressaceae is the largest family of conifers including 29-30 genera, 14 are monotypic. 16 genera are distributed in the Northern hemisphere, 12 in the Southern hemisphere, and 2 in both hemispheres. Of the Northern taxa, *Cupressus* L., *Chamaecyparis* Spach. and *Platycladus* Spach. Some of the Southern taxa are *Callitris* confined to Australia, Tasmania, and New Caledonia, *Libocedrus* to New Zealand and New Caledonia, and *Widdringtonia* to south Africa. Altogether forming about 135 species in which *Juniperus* 53 spp., *Cupressus* 15 spp., and *Callitris* 15 spp. are the largest genera (Farjon, 2010; Farjon and Filer, 2013).

According to the flora of Iraq, *Juniperus oxycedrus* is naturally distributed in Iraq, commonly in North–West Forest zone. Several introduced Cupressaceae taxa are widely planted in parks, roadsides, resorts, and urban areas, especially *Cupressus sempervirens*, *Cupressus arizonica,* and *Platycladus orientalis* (Guest and Townsend, 1966; Shahbaz, 2010). Both *C. sempervirens* and *J. oxycedrus* considers endemic in the Mediterranean region. But, due to substantial climate changes, human horticultural activities, and interfering natural environments the exact natural range of *C. sempervirens* is ambiguous. Yet, North Iran, Syria, Lebanon, Turkey, and Greece are supposed to be the natural habitat (Earle, 2021; Vidakovic, 1991). *J. oxycedrus* natural distribution range is from eastern regions of the Mediterranean to Caucasus mountains and Iran also inland parts of western Asia and Europe at elevation range between 0 – 1400 meters above sea level (asl) (Jovanoviæ, 1986). *Platycladus orientalis* is native to China, Koria, and east Russia. However, it is widely distributed and naturalized in Asia, Europe, and North America at an altitude of 300-3,300m. (Farjon, 2005; Fu et al., 1999). *C. arizonica* is native to the United States and Mexico at altitudes 750-2200m. (Eckenwalder, 2020; Vidakovic, 1991). All these species are considered tolerant to summer hot dry conditions, and also adapted to winter drought frizzing temperatures ranging between (-12.2° C to -17.7°C) (Bannister et al., 2001). Anatomical data had long application history in taxonomy, they help in clarifying interrelationships above the species level, identifying damaged or sparse plant material, and identification of herbarium materials (Radford et al., 1974). Furthermore, with the assistance of new technologies, anatomical features can be put into action for palaeobotanical taxonomy (Escapa et al., 2016). Modern conifers appeared in the Triassic period with Podocarpaceae and Araucariaceae. Still, some of their characters had hardly or did not change, including wood anatomy. Because the wood anatomy of conifers is fairly simple and hard to distinguish among families (Condamine et al., 2020; Farjon, 2010).

In general, the basic structure of many conifers leaves is quite similar but varies in detail (Bercu et al., 2010). Many conifers belong to temperate climate zones, which is why the leaves of conifers developed xeromorphic structures to be proof against water loss in summers and low water content in the soil during snowfalls (Crang et al., 2018). Lakušić and Lakušić (2011) suggest that the driving force behind leaf anatomical character differentiation is mainly the phylogeny of taxa, also but to less degree the ecological factors.

Anatomical studies on gymnosperms in the Kurdistan region are scares, whereas comparative anatomical studies on Cupressaceae family lakes are almost entirely. This research aims to describe and shed light on the leaf anatomy of five Cupressaceae taxa (*C. sempervirens* var. *horizontalis, C. sempervirens* var. *pyramidalis, C. arizonica, P. orientalis, and J. oxycedrus*) while exploring the possibility of acquiring anatomical characters that help in taxonomical differentiation.

2. MATERIAL AND METHODS

Fresh mature leaves were collected from gardens and natural growing areas of the Duhok, Irbil, and Sulaymaniyah. The

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* Corresponding author

procedure of slide preparation was conducted according to the procedure described by Najmaddin and Mahmood (2016).

Firstly, samples of mature leaves were fixed in (Formaldehyde, 5%, acetic acid, 5%, 70% alcohol, 90%) Then, Plant materials were dehydrated and cleared using a series of alcohol and xylene. For the paraffine Infiltration process, dehydrated samples were embedded in a series of melted paraffine and xylene. Next, plant samples were embedded in paraffin, and blocks were prepared using a metal mold. Using a microtome, 10 µm slices were made and fixed on slides. Then, the slides underwent a series of staining steps using ethanol, xylene, distilled water, safranin, and light green. cover slides with Dibutylphthalate Polystyrene Xylene (DPX), and place a cover slide.

For each studied species, several descriptive characters, in addition to the mean, range, and standard deviation of numerical characters were calculated. Many slides were photographed and studied with the help of light microscopes (Zeiss Axiostar plus and XDS-1M inverted microscopes) equipped with a 10X Dino-Eye USB Microscope Camera (AM4023X) and ImagJ measurement program version 1.53.

3. RESULTS AND DISCUSSIONS

The cross-section of leaves midrib was observed. The results of the anatomical characters studied are shown in the comparative Tables (1 and 2). As expected by the authors, studied leaves clearly showed xeromorphic properties. For example, the cuticle is thick on both surfaces, particularly the surface that exposes a higher percentage of sunlight, which means the adaxial face of *J. oxycedrus* and abaxial face of *Cupressus* and *Platycladus* taxa. The main advantage of a thick waxy cuticle is to reduce heat, water transpiration, and salinity (Riederer and Schreiber, 2001).

3.1 leaf cross-section

The leaf cross-section of *Cupressus* taxa contains two united oblong main scales with two triangle lateral scales, providing an elliptic shape. *P. orientalis* is made up of a stem with two leaf scales, producing a widely rhombic outline (Figure 1.D). *J. oxycedrus* outline is simple and has the shape of an inverted triangle with a prominence separating two depressions, also known as stomatal band (Farjon, 2010) (Figure 1. E).

3.2 Epidermis

This outer epidermal layer is made up of cuticle and epidermal cells. Cuticle surrounds the entire leaf surface. Generally, it is thick and penetrates epidermal cell interspaces. Abaxially, the mean thickness varies slightly among *Cupressus* taxa (6.8 – 7.7 µm) but declines to (5.4 µm) in *J. oxycedrus*, down to (4.4 µm) in *P. orientalis*. Adaxially, the cuticle thickness declined in all taxa, still *C. sempervirens* var. *horizontalis, C. sempervirens* var. *Pyramidalis* and *C. arizonica* have a higher thickness (5.3, 5, 4.9 µm.) respectively. while *P. orientalis* has the lowest value (2.2 µm). The outline of the cuticle gives the pattern either entire-slightly lobed as for the abaxial surface of *C. sempervirens* var. *horizontalis, C. sempervirens* var. *Pyramidalis*, also both surfaces of *P. orientalis* (Figures 2.C, 3.B, 5.D); Or conspicuously lobed-papillated pattern as in *C. arizonica, J. oxycedrus*, and the adaxial surface of *C. sempervirens* species (Figure 6.A).

Epidermal cell forms a single layer of thick-walled parenchyma cells. The shape is mostly rounded or elongated; however, the cell wall may prominence abaxially, providing an irregular shape. In general, the upper surface cells have greater cell thickness than the lower surface, except in *C. sempervirens* var. *Pyramidalis*. *C. arizonica* is distinct, because the thickness at both surfaces is nearly equal, also significantly higher than other taxa. remained plants *C. sempervirens var. horizontalis, P. orientalis, and J. oxycedrus* have various abaxial-adaxial thicknesses (12.5-14.1, 14.4-15.8, 10.8-12.7) respectively (Table 1).

Stomata in all studied taxa are deeply sunken, with papillose subsidiary cells that arched over the stomatal cavity and a small cavity below the guard cells (Figures 2.D, 3.E, 4.D, 5.B, 6.C). In the *Platycladus* orientalis, stomata are amphistomatic (can be found on both leaf faces), either on the main scales or the stem. Adaxially they are abundant. On the other side, abaxial stomata are few and found infrequently. *J. oxycedrus* stomata are epistomatic (found only adaxially), abundantly laying at both upper face's depressions (stomatal band) where generally few epidermal cells separate them (Figure 6.C). *Cupressus* taxa are also epistomatic, where stomata exist in fissures between leaves (Table 2).

Stomata appear more frequently adaxially, at fissures between *Cupressus* and *Platycladus* taxa leave, while being fewer in number and scattered at the abaxial surface of *P. orientalis*, which helps in reducing water loss (Pirasteh-Anosheh et al., 2016). Resembling previous anatomical studies (Fouda, 2004; Khan et al., 2019), abaxial stomata did not been found in *Cupressus* taxa. Although morphological studies declare their existence. This could be a result of their few numbers, and the scattered distribution near the leaves' base (Farjon, 2010). So, when the leaves are been sliced at the midrib, stomata are not included in sections.

3.3 Hypoderm

The hypoderm is a layer of sclerenchyma cells beneath epidermal cells that provide mechanical support. This is another xeromorphic adaptation, which also helps in water retention, alongside supporting leaf structure (Beck, 2010; Cutler et al., 2008). The supportive character may explain the greater thickness of hypoderm in *J. oxycedrus* with the presence of a sclerenchyma cell layer beneath the vascular bundle since the leaves are much bigger compared to other investigated species (Figure 6.A,E) (San-Miguel-Ayanz et al., 2016).

In all taxa, the hypoderm appears as a single layer of highly lignified cells with a small lumen; except for leaf edges (corners), where the hypoderm may form multiple layers. Also, in *C. arizonica* where it may increase up to 2 layers (Figure 4.B). The shape varies from angular, elongated, or irregular cells. hypoderm existence is restricted to the abaxial part of the leaf, except for *J. oxycedrus*, where it presents as a continuous layer on both leaf surfaces, except when broken by stomatal bands or the resin duct. The thickness varies greatly among species. While *C. sempervirens* var. *horizontalis* has the thinnest hypoderm (6.9 µm), other taxa have variable thickness, (14.9µm) for *P. orientalis*, (12.1µm, 10.5µm) for *C. sempervirens* var. *Pyramidalis,* and *C. arizonica* respectively. *J. oxycedrus* has the thickest layer (22.1 µm) adaxially and (27.8 µm) abaxially (Table 1).

3.4 Mesophyll

Mesophyll is differentiated into palisade cells and spongy tissue. In the parts of the leaf that is exposed to light, palisade cells form a single layer beneath the hypoderm perpendicularly to the epidermis. This comprise abaxial surface of *C. sempervirens* var. *horizontalis*, *C. sempervirens* var. *Pyramidalis*, *C. arizonica*, *P. orientalis,* and both surfaces of *J. oxycedrus* (Figures 2C, 3.D, 4.B, 5.D, 6.B). Although the shape of cells is elliptic, in leaf parts exposed to the highest portion of sunlight the shape may become conical where the widest end is just below the hypoderm. This shape change is to increase light absorption sufficiency (Crang et al., 2018).

Table 1: Anatomical traits comparison of the leaves in the studied taxa in µm.

Taxon / Character		*C. sempervirens* var. *horizontalis*		*C. sempervirens* var. *Pyramidalis*		*C. arizonica*		*P. orientalis*		*J. oxycedrus*	
		Abaxial	**Adaxial**	**Abaxial**	**Adaxial**	**Abaxial**	**Adaxial**	**Abaxial**	**Adaxial**	**Abaxial**	**Adaxial**
Cuticle thickness	Mean	7.7	5.3	6	5	6.8	4.9	4.1	2.2	5.4	6.7
	Range	9.9-5.9	7.7-3.7	9.4 - 4.4	7.5 - 3.3	8.6 - 3.7	8.5 - 2.5	5.8 - 2.9	3.5 - 1.5	7.6 - 3.7	8.7 - 5.0
	S.D.	1.1	1	1.1	1.1	1.3	1.8	0.7	0.5	1	1.1
Epidermal layer thickness	Mean	12.5	14.1	15.2	13.9	20	19.9	14.4	15.8	10.8	12.7
	Range	15.1-9.5	17.8-10.2	18.1 - 12.2	17.2 - 10.6	23.3 - 16.3	24.4 - 16.6	16.7 - 12.6	17.9 - 13.4	13.3 - 8.6	17.1 - 9.5
	S.D.	1.6	2.2	1.7	1.8	2.2	2.2	1.1	1.2	1.2	2.2
Palisade Cells		**Height**	**Width**	**Height**	**Width**	**Height**	**Width**	**Height**	**Width**	**Height**	**Width**
	Mean	46.6	16.1	59.1	17	91.8	27	48.6	17.1	52.8	26.1
	Range	63.2 - 31.4	22.6 - 10.6	79.9 - 35.5	23.6 - 11.1	117.2 - 61.4	36.1 - 20.2	68.7 - 31.1	23.0 - 12.8	70.9 - 40.7	35.9 - 18.0
	S.D.	8.1	3	12.8	2.9	16.2	4.3	9.8	2.7	7.6	4.4
Hypodermal layer thickness										**Adaxial**	**Abaxial**
	Mean	6.9		10.5		12.1		14.9		22.1	27.8
	Range	9.6 - 4.3		14.8 - 7.6		17.0 - 7.8		21.2 - 9.2		32.2 - 12.7	35.6 - 18.2
	S.D.	1.5		2.1		2.3		3.5		4.7	4.9
Xylem thickness	Mean	37.3		20		42.7		50.2		42.7	
	Range	50.5 - 24.3		29.0 - 14.4		60.8 - 28.1		84.4 - 25.4		61.7 - 22.3	
	S.D.	7.2		4		8.2		10.3		7.2	
Phloem thickness	Mean	45.6		32.3		55.7		52.7		46.9	
	Range	57.5 - 26.9		47.5 - 23.8		76.3 - 30.8		74.9 - 37.2		59.7 - 35.8	
	S.D.	7.1		6.5		12.2		11.2		7.1	
Resin Duct diameter	Mean	69.5		79.9		125.4		78.1		44.5	
	Range	93.0 - 49.5		104.1 - 62.3		247.6 - 46.6		118.3 - 46.1		55.4 - 35.6	
	S.D.	13.7		16.1		29.8		19.5		5.5	

Table 2: Anatomical comparison of the leaf traits in the studied taxa.

Species	**Stomata**	**Palisade parenchyma**	**Secretory canals**	**Endodermis layer surrounds Secretory canals**
C. sempervirens var. *Horizontalis*	Adaxial Surfaces	Abaxial Surface	Small, centrally located, one per each lateral leaf	2
C. sempervirens var. *Pyramidalis*	Adaxial Surfaces	Abaxial Surface	Small, centrally located, one per each lateral leaf	2
C. arizonica	Adaxial Surfaces	Abaxial Surface	Largest among taxa, shifted toward leaf's abaxial surface, one per each lateral leaf	2-3
P. orientalis	Both Surfaces	Abaxial Surface	Small, shifted toward leaf's abaxial surface, centrally located, one per each leaf	1-2
J. oxycedrus	Adaxial Surface	Both Surfaces	Smallest among taxa, situated at the distal edge of the abaxial surface, one per leaf	2-3

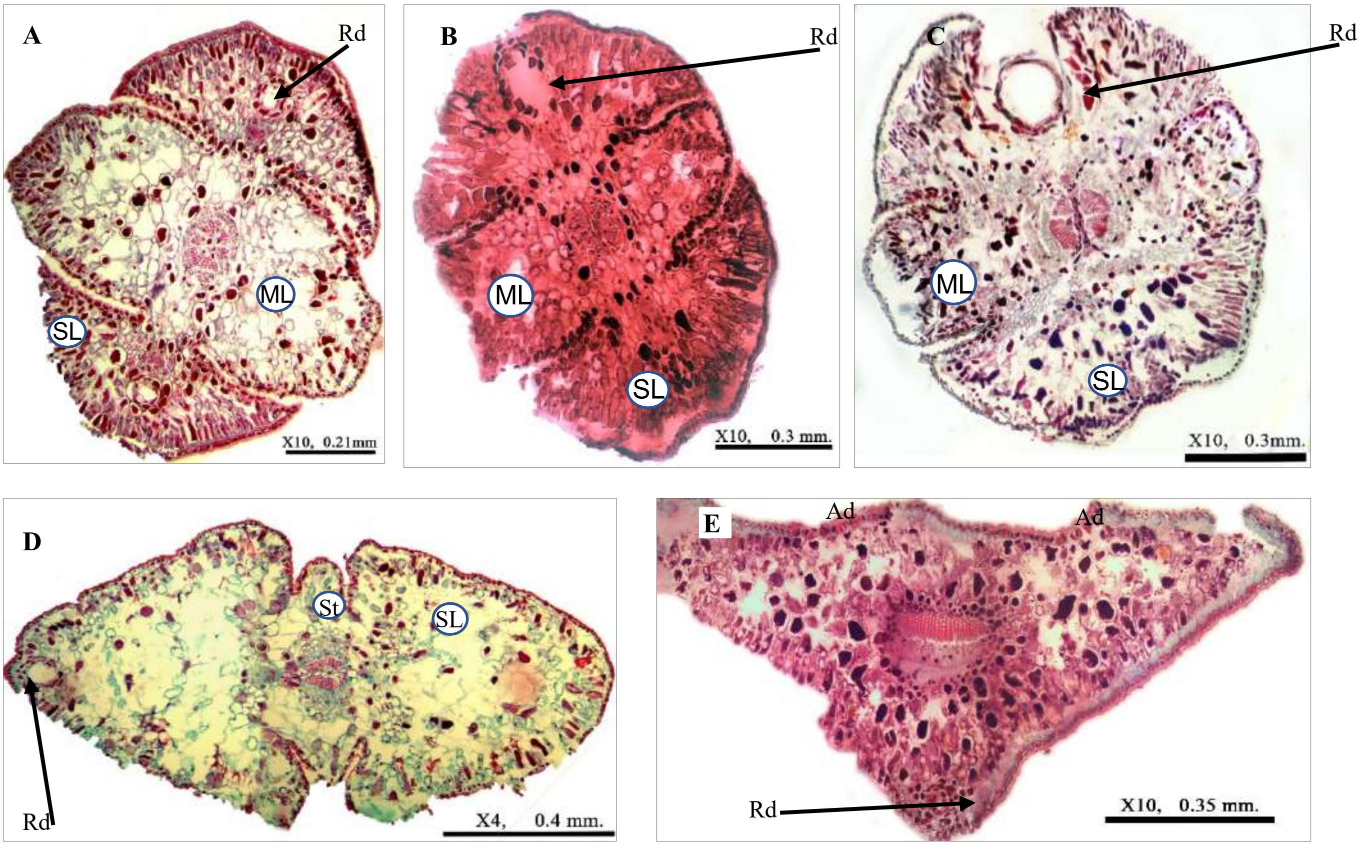

Figure 1: Leaf cross section outline of (A) *Cupressus sempervirens* var. *horizontalis*, (B) *C. sempervirens* var. *Pyramidalis*, (C) *Cupressus arizonica*, (D) *Platycladus orientalis*, (E) *Juniperus oxycedrus*. (ML): Main leaf, (SL): Scale leaf, (St): Stem, (Rd): Resin duct, (Ad): Adaxial depression (stomatal band).

The Dimensions varies, *C. sempervirens* var. *horizontalis*, *C. sempervirens* var. *Pyramidalis* and *P. orientalis* have about the same dimensions (46.6×16.1, 59.1×17.0, 48.6×17.1 µm.) respectively. *J. oxycedrus* has wider palisade cells (52.8-26.1µm.). as for epidermal cells, *C. arizonica* has considerably larger palisade cells (91.8×27µm.). spongy tissue is thin-walled parenchymatous cells with irregular shapes. Both palisade and spongy tissue contained starch granules.

3.5 Transfusion tissue

This tissue can be differentiated from spongy parenchyma by having bordered pits in their wall. This tissue forms around vascular bundles. In both *C. sempervirens* and *C. arizonica*, they mainly occur on right and left sides of vascular bundles of both lateral leaves, but occasionally around the main leaves' phloem (Figures 2.A, 3.F, 4.E). In *C. arizonica* the tissue comprises a larger portion of the lateral leaf. In *P. orientalis* they form as two bundles at leaves bases (Figure 5.C). Again in *J. oxycedrus*, transfusion tracheids laterally form two bundles at both sides of vascular bundles (Figure 6.D).

Transfusion tissues are found exclusively in gymnosperm, they help in storage and transportation. The tissue is made up of short tracheid-like cells mixed with parenchyma cells, with circular bordered pits at their walls. The pitting in this study is Cupressoid-type (HU and YAO, 1981; Leverett et al., 2021). This tissue may not have taxonomic importance by itself, but its relative position to other leaf features can be useful. For example, in *J. oxycedrus*, it is adaxially positioned near xylem tissue within the endoderm, while in other taxa they are free. Furthermore, in *Cupressus* taxa, they are located just below the resin duct, while in *P. orientalis* the distance to the resin duct is significantly greater.

3.6 Vascular bundles

the vascular bundle is consisting of the xylem and phloem tissues. Xylem tissue is made up of thick-walled circular-elliptic tracheids, surrounded by the phloem's angular thin-walled sieve cells, in both *C. arizonica* and *Cupressus sempervirens* taxa. Main leaves share a central circular vascular bundle that is enclosed by a layer of endodermis (Figures 2.B, 3.C, 4.F). The pith is radial parenchyma penetrating xylem, providing mostly polygonal shape. In the lateral leaves, the vascular bundle is open, made up of a bundle of phloem positioned over a bundle of xylem, and centrally located at the lateral leaf base. *P. orientalis* has an elliptic vascular bundle in the stem, split by the pith and surrounded by a layer of endoderm, with a small open vascular bundle between transfusion tracheids in each leaf (Figure 5.A). Unlike other studied species, *J. oxycedrus* vascular bundle is oblong, horizontally suited at the center of the leaf, xylem in adaxial direction and phloem in abaxial direction with a layer of sclerenchyma cell beneath phloem. Here, The Pith is absent, but a layer of endoderm encloses the xylem, phloem, sclerenchyma cell layer, and transfusion tracheids (Figure 6.E). the size of the xylem - phloem varies among variety and species. Largest vascular occurs in *P. orientalis* (50.2-52.7 µm.), and smallest in *C. sempervirens* var. *pyramidales* (20-32.3 µm.). other taxa have various sizes, *C. sempervirens* var. *horizontalis* (37.3-45.6 µm.), *C. arizonica* (42.7-55.7 µm.), and *J. oxycedrus* (42.7-46.9 µm.) (Table 1).

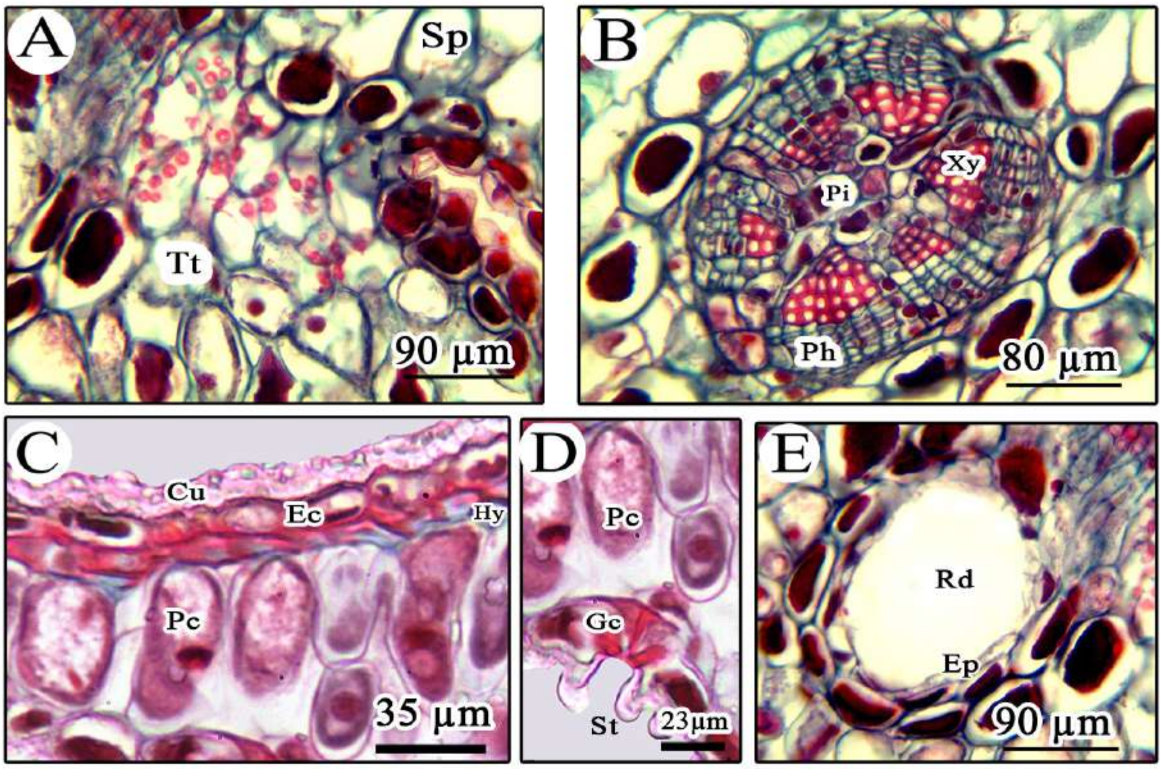

Figure 2: Leaf anatomical features of *Cupressus sempervirens* var. *horizontalis*. (A) (Tt): Transfusion tracheids, (Sp): Spongy parenchyma; (B) (Pi): Pith, (Xy): Xylem, (Ph): Phloem; (C) (Cu): Cuticle, (Ec): Epidermal cells, (Hy): Hypoderm, (Pc): Palisade cells; (D) (St): Stomata, (Gc): Guard cells; (E) (Rd): Resin duct, (Ep): Epithelial cells. All are photographed at X40 magnification.

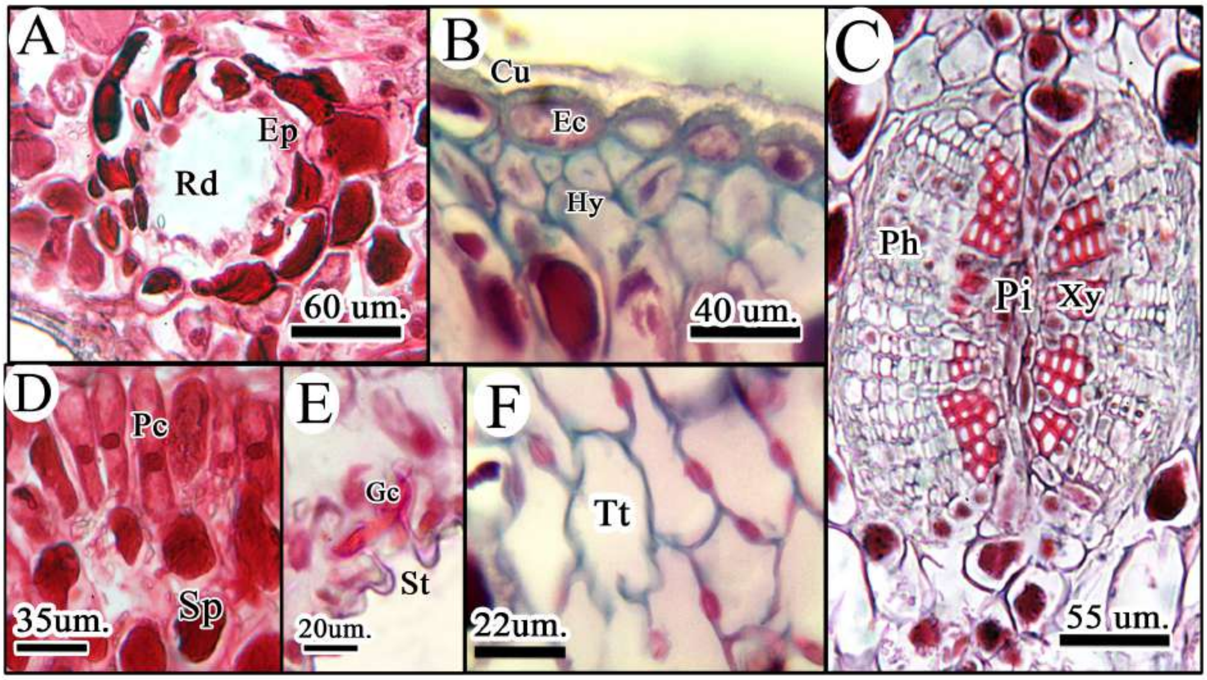

Figure 3: Leaf anatomical features of *Cupressus sempervirens* var. *pyramidalis*. (A) (Rd): Resin duct, (Ep): Epithelial cells; (B) (Cu): Cuticle, (Ec): Epidermal cells, (Hy): Hypoderm; (C) (Pi): Pith, (Xy): Xylem, (Ph): Phloem; (D) (Pc): Palisade cells, (Sp): Spongy parenchyma; (E) (St): Stomata, (Gc): Guard cells; (F) (Tt): Transfusion tracheids. Features are photographed at X40 magnification, except transfusion tracheids (X100**)**.

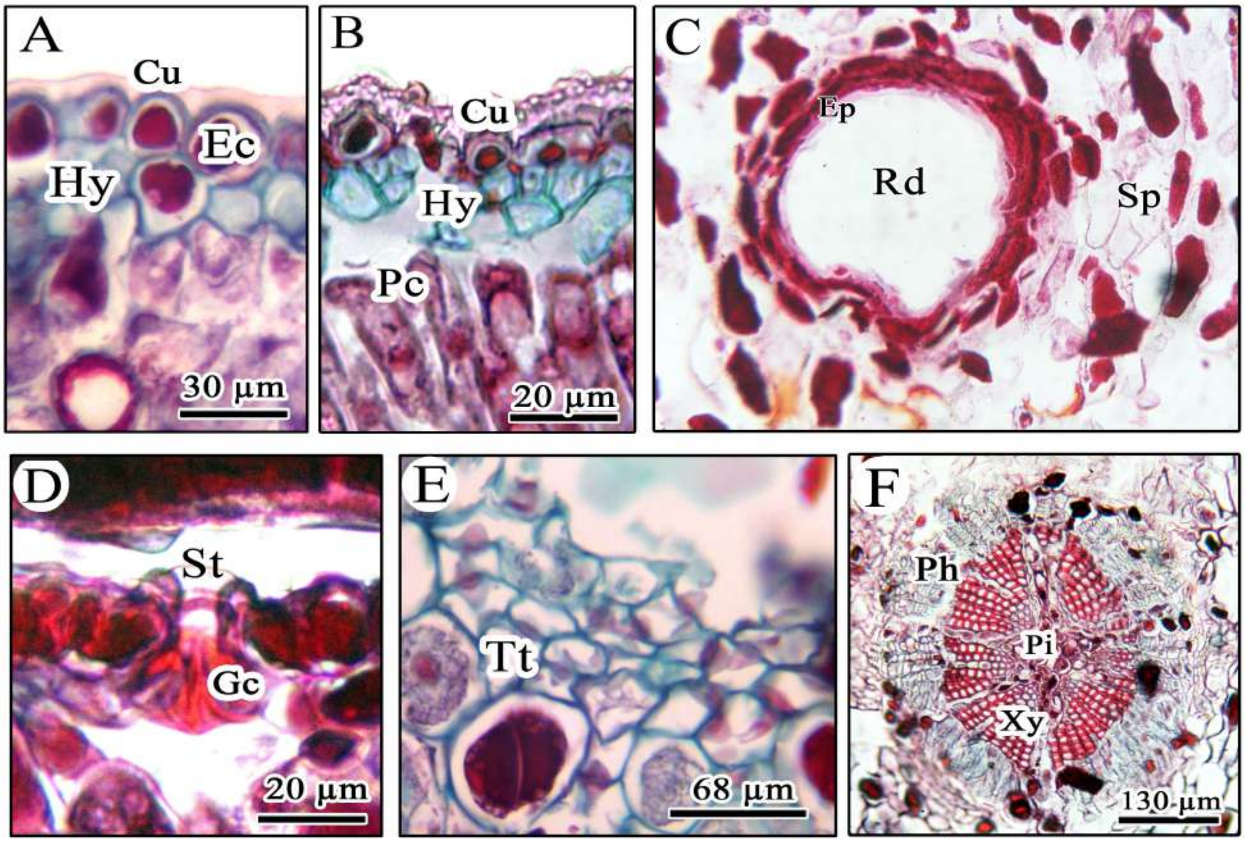

Figure 4: Leaf anatomical features of *Cupressus arizonica*. (A) (Cu): Cuticle, (Ec): Epidermal cells, (Hy): Hypoderm; (B) (Cu): Cuticle, (Hy): Hypoderm, (Pc): Palisade cells; (C) (Sp): Spongy parenchyma, (Rd): Resin duct, (Ep): Epithelial cells; (D) (St): Stomata, (Gc): Guard cells; (E) (Tt): Transfusion tracheids; (F) (Pi): Pith, (Xy): Xylem, (Ph): Phloem. Features are photographed at X40 magnification.

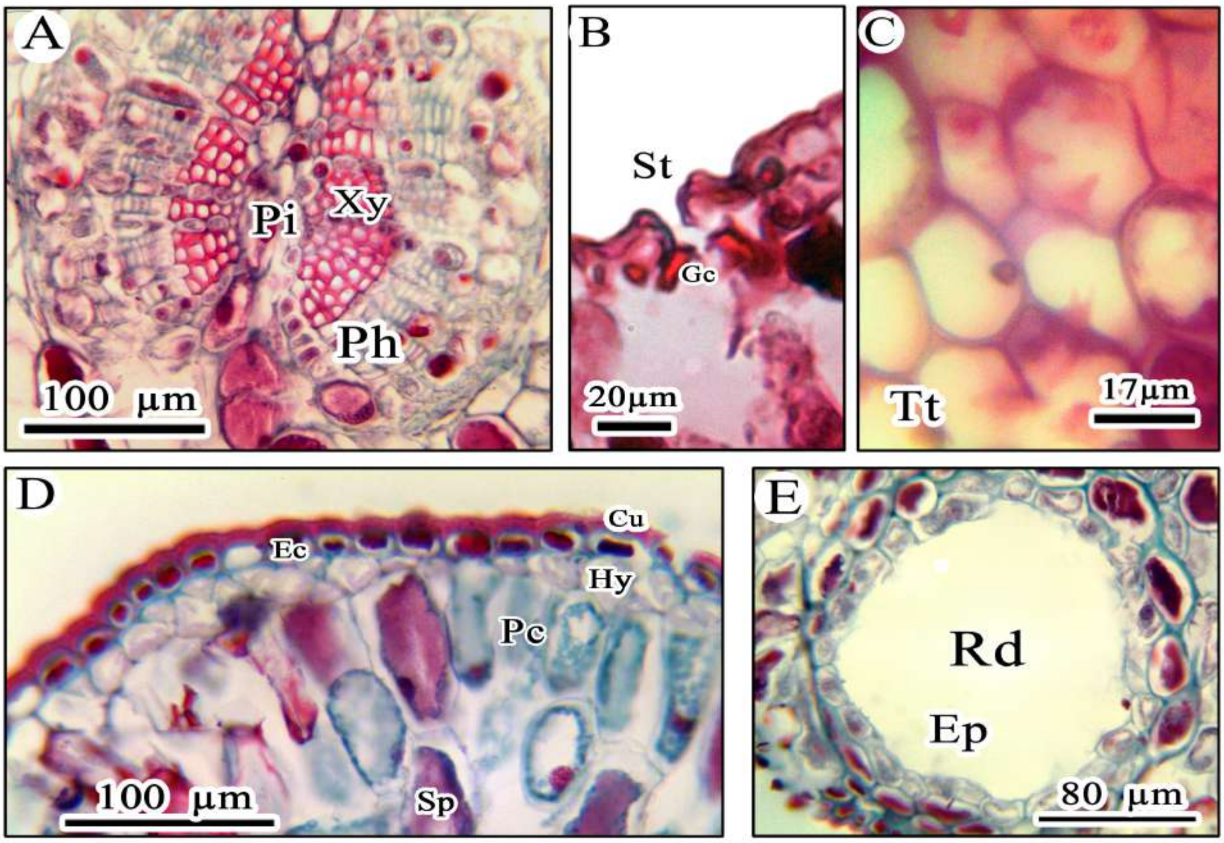

Figure 5: Leaf anatomical features of *Platycladus orientalis*. (A) (Pi): Pith, (Xy): Xylem, (Ph): Phloem; (B) (St): Stomata, (Gc): Guard cells; (C) (Tt): Transfusion tracheids; (D) (Sp): Spongy parenchyma, (Cu): Cuticle, (Ec): Epidermal cells, (Hy): Hypoderm, (Pc): Palisade cells; (E) (Rd): Resin duct, (Ep): Epithelial cells. All features are photographed at X40 magnification, except transfusion tracheids (X100).

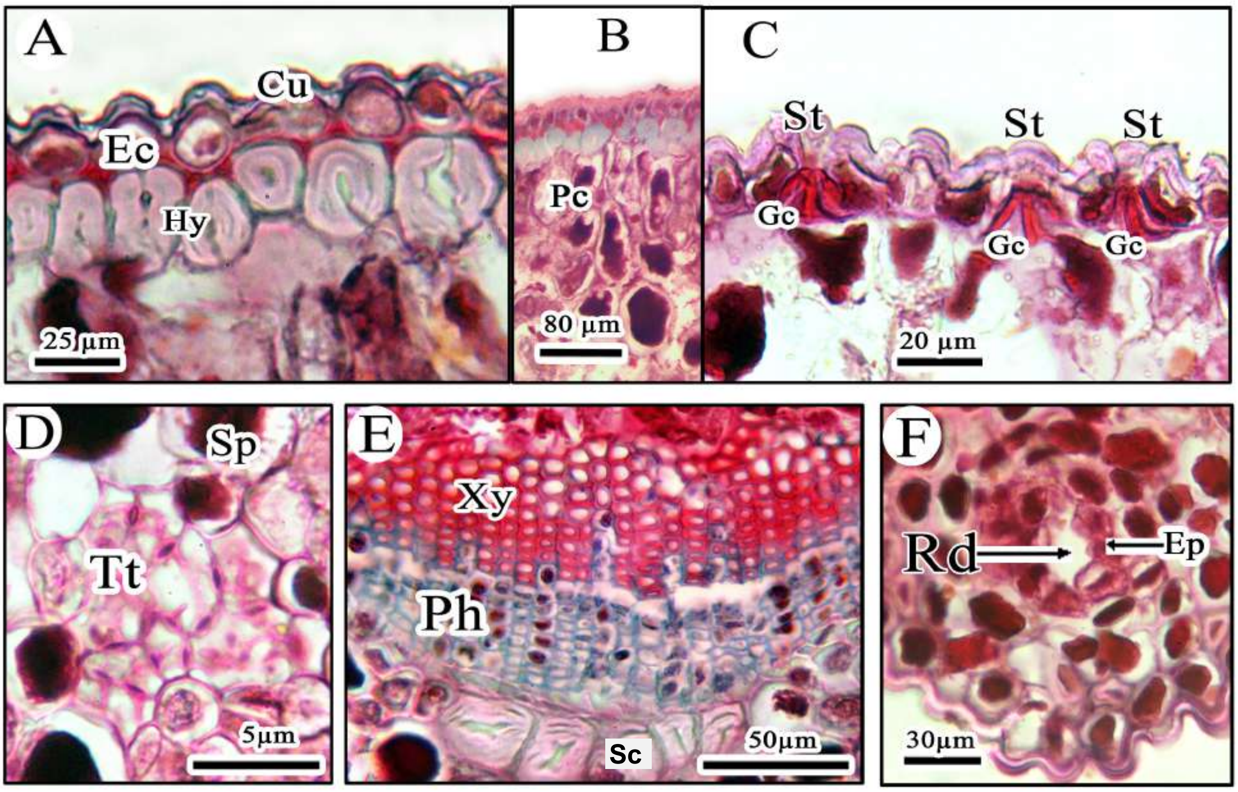

Figure 6: Leaf anatomical features of *Juniperus oxycedrus*. (A) (Cu): Cuticle, (Ec): Epidermal cells, (Hy): Hypoderm; (B) (Pc): Palisade cells; (C) (St): Stomata, (Gc): Guard cells; (D) (Tt): Transfusion tracheids, (Sp): Spongy parenchyma; (E) (Xy): Xylem, (Ph): Phloem, (Sc): Sclerenchyma cells; (F) (Rd): Resin duct, (Ep): Epithelial cells. Features are photographed at X40 magnification, except palisade cells X10.

3.7 Secretory canal (Resin duct)

Resin ducts also provide sufficient information for species differentiation. Their size, number of endodermis layers, and position, all varied among species (Table 1 and 2). This structure was founded in all examined taxa as one layer of thin-walled epithelial cells lined within the endoderm as one canal per each leaf including *J. oxycedrus*, in the *Cupressus* and *Platycladus* taxa found as one canal per each lateral leaf. Canals of *C. sempervirens* varieties are centrally positioned, the diameter is small (69.5-79.9 µm.), enclosed by 2 layers of endoderm (Figures 2.E, 3.A). *P. orientalis* also had small canals (78.1 µm.) that was abaxially positioned at the leaves edge, with 1-2 endoderm layers enclosing it. *C. arizonica* has the largest secretory canal (125.4µm.), that abaxially located near the leaf margin with 2-3 layers of endoderm. The smallest secretory canal found in *J. oxycedrus* (44.5µm) is inside 2-3 layers of endoderm. These abaxial resin canals may cause the hypoderm to be discontinued in *J. oxycedrus, P. orientalis,* and *C. arizonica*. (Figures 1.E, 6.F). The obtained results in this study relating to the type and distribution of anatomical features (epiderm, hypoderm, vascular bundle) correspond to previous anatomical studies (Hamidpour et al., 2011; HU and YAO, 1981; Koçyiğit and Tümer, 2017; Yao and Hu, 1982; Zhou et al., 2019).

4. CONCLUSION

Although studied taxa showed significant uniformity in leaf basic structure, the number of variations in the details suggest that the anatomical characteristics are feasible to classify Cupressaceae at species and genera levels. Cuticle characters (adaxial and abaxial thickness, ornaments) are almost corresponding at the genera level. However, epidermal cell thickness and palisade dimensions were found to be correlated to species. Some characters can provide identification keys. For example, the shape of the leaf cross-section outline of *Cupressus* taxa differs from *J. oxycedrus* and *P. orientalis*. Also, the lack of pith, palisade cell distribution, characters of vascular bundle, and a layer of highly lignified sclerenchyma cells in *J. oxycedrus*. Moreover, the thickness of epiderm, sizes of palisade cells, and risen canal diameter in *C. arizonica* were the highest among the studied species. However, *C. sempervirens* varieties did not show any key differences.

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