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Morphological, anatomical and palynological studies on *Helichrysum plicatum* DC. subsp. *plicatum* DC. and *Helichrysum plicatum* DC. subsp. *polyphyllum* (Ledeb.) (Asteraceae).

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Abstract

In this study, *Helichrysum plicatum* subsp. *plicatum* and *Helichrysum plicatum* subsp. *polyphyllum* (Asteraceae) represented in the genus *Helichrysum* were investigated for the taxonomical purposes. The specimens belonging to both taxa were collected different locations from Turkey and investigated in view of morphological, morphometrical, anatomical and palynological features to determine the similarity and differences between two taxa. Anatomical studies were carried out on the stem and root characters with the Light microscope and illustrated. Pollen features of both taxa were observed with the Light and Scanning Electron Microscopy (SEM). Both taxa were found different in length of internodes, cauline leaves leaf apices, phyllaries apices, phyllaries shapes of the upper and the lower facial hairs features from each others. The plants have woody root anatomy and the stem is herbaceous. The pollen type of both taxa were determined as tricolporate, *Helichrysum plicatum* subsp. *plicatum* pollen shape was spheroidal as *Helichrysum plicatum* subsp. *polyphyllum* pollen shape was oblate-spheroidal and reticulate pollen ornemantation was observed. Consequently, there are some morphological, morphometrical and palynological differences in both taxa, however, no significant differences were determined in view of anatomical features. The findings obtained from the study were discussed with each other and the genus patterns.

Keywords: *Helichrysum*, Asteraceae, Morphology, Anatomy, Pollen.

1. Introduction

The *Helichrysum* Gaertner species are also found in southern Europe, south-west Asia, southern India, Sri Lanka and Australia, most species occur in Africa, including Madagascar (Lourens et al. 2008). This genus which is represented by 24 species, 30 taxa of which, 17 are endemic, has been recorded in the Flora of Turkey (Guner, 2012).

The name of the plant, from the Greek "helios", sun and "chryos", gold, relates to the typical bright yellow coloured inflorescences which represent the drug (Chinou et al. 1996, 1997; Perrini et al. 2009).

Species from the genus *Helichrysum* are widely used as traditional medicine to cure various ailments (Hutchings & Van Staden 1994; Lourens et al. 2008; Sezik et al. 2001). In Turkey, several *Helichrysum* spp. are used in folk medicine for removing the kidney stones and as diuretics. The diuretic and bile regulatory effects of the *Helichrysum* spp. are due to the flavonoids they contain (Cubukcu 2002; Suzgec et al. 2011).

H. plicatum subsp. *plicatum* and *H. plicatum* subsp. *polyphyllum* have been described in Flora of Turkey and East Aegean Islands (Davis and Kupicha, 1975).

In this study the taxons are discussed through the direction of the literature results and introduced according to the morphologically, anatomically and palynological. There is not any study in the literature related with *H. plicatum* subsp. *plicatum* and *H. plicatum* subsp. *polyphyllum* which is encountered about the morphology and anatomy.

2. Materials and Methods

2.1. Morphological studies:

The aerial and underground parts of *Helichrysum* taxa used in this study were collected in the flowering season from the different regions of Turkey. The research material was collected from the locations shown in Table 1. The taxonomic description of the plant was made according to Davis and Kupicha(1975) and also confirmed by the herbarium samples of the examined taxa in ANK, HUB and GAZİ.

2.2. Anatomical studies:

The materials necessary for anatomic studies were protected in 70% alcohol and cross-sections taken from by hands the plant's roots and stems were analyzed. Photographs from the preparations were taken with a camera adapted to a Olympus BX 51 microscope.

2.3. Palynological studies:

Pollen materials were removed from living specimens in the field. Data on size were based on the measurements of 50 pollen grains which used Wodehouse method and light microscopy. After pollen

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grains were coated with a thin layer of gold/palladium for 3 min with an Denton sputter coater, they were examined with a JEOL JSM 7001F SEM at the department of physics of Firat University, Elazig, Turkey.

Table 1. Locations of *Helichrysum* taxa.

Taxa	Locations
H. plicatum subsp. *plicatum*	B4-Malatya: Malatya between Gürün, road side-1400 m.,21.VI.2010, *Ö.Elkıran* 1005.
H. plicatum subsp. *polyphyllum*	C3-Isparta: Aksu mountain- 2000 m.,22.VI.2010, *Ö.Elkıran* 1018.

3. Results and Discussion

Morphological properties:

The morphological properties determined in our study are generally similar to those in the Flora of Turkey (Davis and Kupicha, 1975). In this study, some additional new characters were added to Flora of Turkey. These characters are; throughout the plant, the root type, basal leaves, apices, margin, base, type of the upper facial and lower facial hairs, length of internodes, cauline leaves margin, apices, type of the upper facial and lower facial hairs, piece of flowers in capitulum, phyllaries lenght, shape, margin, apices, type of the upper facial and lower facial hairs, tubular flowers colour, length, hair condition. Morphological and morphometric characters of *H. plicatum* subsp. *plicatum* and *H. plicatum* subsp. *polyphyllum* findings are given in Table 2.

***H. plicatum* subsp. *plicatum*:** Plant perennial, herbaceous or suffruticose, glandular, taproot, throughout the plant, including capitula 8.5-21.5 cm, except capitula 7-19 cm. Basal leaves 1-10 x 10-100 mm, linear-oblanceolate, apices acuminate, margin continuous, base obtuse, shapes of the upper facial and lower facial hairs tomentose, attachment subamplexicaul. Stem erect or curved, 4-42 cm, branched, subglabrose to lanate tomentose or lanate, internod 3-10 mm, cauline leaves 1-5 x 10-40 mm, linear oblanceolate to linear or acute, margin continuous, apices obtuse, shapes of the upper facial and lower facial hairs lanate, attachment subamplexicaul, grayish-green. Capitula subglobose, 4-9 mm, borne 5-9 in dense corymbs. Phyllaries 1.5-2 x 2-3 mm, oblanceolate to spatulate, base obtuse to acute, margin continuous, apices acute, shapes of the upper facial and lower facial hairs pilosus, loosely imbricate, yellow. Tubular flowers color from yellow to apricot, 3-4 mm, tomentose. Flowering time is between June-August (Figure 1).

Distribution of the taxa in Turkey: Bursa, Bolu, Zonguldak, Amasya, Sivas, Trabzon, Erzurum, Kars, Kütahya, Konya, Ankara, Kayseri, Maraş, Erzincan, Bitlis, Van, Ağrı, Antalya, Isparta, Niğde, Hatay, Hakkari (Figure 2) (Davis and Kupicha, 1975; Tubives, 2013).

a b

Figure 1.(a) Herbarium sample of *H. plicatum* subsp. *plicatum*

Figure 1.(b) General apperance of *H. plicatum* subsp. *plicatum*, **a.** General apperance, **b.** Cauline leave, **c.**Phyllary, **d.** Flower, **e.** Achene

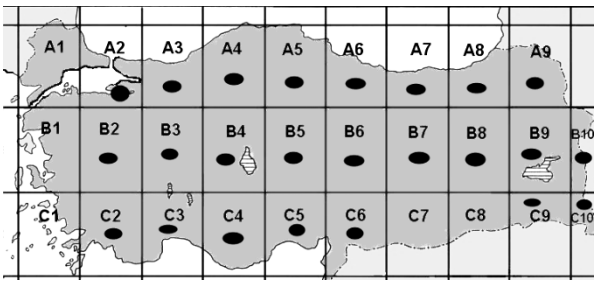

Figure 2. Distribution of *H. plicatum* subsp. *plicatum* inTurkey (Tubives, 2013)

***H. plicatum* subsp. *polyphyllum*:** Plant perennial, herbaceous or suffruticose, glandular, taproot, throughout the plant, including capitula 22-45 cm, except capitula 19-41 cm. Basal leaves 1-2 x 10-100 mm, linear-oblanceolate, apices obtuse, margin continuous, base obtuse, shapes of the upper facial and lower facial hairs tomentose, attachment subamplexicaul. Stem errect, 4-42 cm, branched, subglabrous to lanate-tomentose or tomentose, internod 15-27 mm, cauline leaves 1-20 x 17-70 mm, linear-oblanceolate to linear, margin continuous, apices acute-obtuse, shapes of the upper facial and lower facial hairs tomentose, attachment subamplexicaul, yellowish-green. Capitula spherical to hemispherical, 4-9 mm, borne 4-9 in lose corymbs. Phyllaries 1-2 x 4-6 mm, oblanceolate-spatulate, base obtuse to acute, margin continuous, apices obtuse, type of the upper facial and lower facial hairs tomentose, loosely imbricate, yellowish-green. Tubular flowers apricot-colored, 3 - 3.5 mm, tomentose. Flowering time is between June-August (Figure 3).

Distribution of the taxa in Turkey: Gümüşhane, Kayseri, Maraş, Tunceli, Bingöl, Bitlis, Van, Antalya, Isparta, Hatay, Hakkari (Figure 4) (Davis and Kupicha, 1975; Tubives, 2013).

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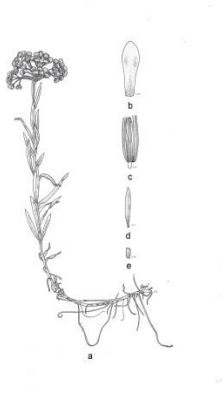

b

Figure 3.(a) Herbarium sample of *H. plicatum* subsp. *polyphyllum*

Figure 3.(b) General apperance of *H. plicatum* subsp. *polyphyllum*, a. General apperance, b. Phyllary, c. Flower, d. Cauline leave, e. Achene

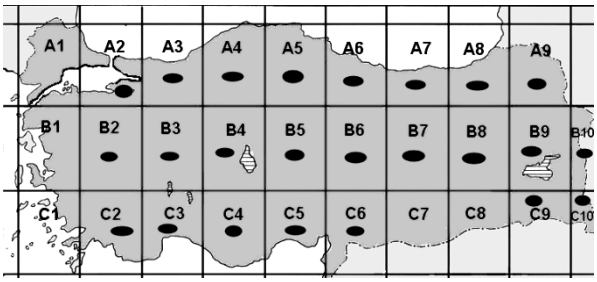

Figure 4. Distribution of *H. plicatum* subsp. *polyphyllum* in Turkey (Tubives, 2013)

Table 2. Morphological and morphometric characters of *H. plicatum* subsp. *plicatum* and *H. plicatum* subsp. *polyphyllum* in Flora of Turkey and Present study.

		H. plicatum subsp. *plicatum*		*H. plicatum* subsp. *polyphyllum*	
Characteristics		**Flora of Turkey**	**Present study**	**Flora of Turkey**	**Present study**
Way of life		Perennial, herbaceous or suffruticose	Perennial, herbaceous or suffruticose	Perennial, herbaceous or suffruticose	Perennial, herbaceous or suffruticose
Throughout the plant (including capitula)		*	8.5-21.5 cm	*	22-45 cm
Throughout the plant (except capitula)		*	7-19 cm	*	19-41 cm
The root type		*	Taproot	*	Taproot
Basal leaves	**Lenght**	2-10 x 10-100 mm	1-2 x 10-13 mm	2-10 x 10-100 mm	1-2 x 10-45 mm
	Leaf shapes	Linear-Oblanceolate	Linear- Oblanceolate	Linear-Oblanceolate	Linear- Oblanceolate
	Leaf apices	*	Acuminate	*	Obtuse
	Leaf margins	*	Entire	*	Entire
	Leaf base	*	Obtuse	*	Obtuse
	Shapes of the upper facial hairs	*	Tomentose	*	Tomentose
	Shapes of the lower facial hairs	*	Tomentose	*	Tomentose
	Leaf attachment	*	Subamplexicaul	*	Subamplexicaul
Stem	**Forms**	Erect or rarely oblique	Curved	Erect or rarely oblique	Erect
	Lenght	4-42 cm	7-17 cm	4-42 cm	20-28 cm
	Branching	Branched	Branched	Branched	Branched

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	Hair condition	Subglabrous to lanate-tomentose	Tomentose	Subglabrous to lanate-tomentose	Tomentose
	Length of internodes	*	3-10 mm	*	15-27 mm
Cauline leaves	**Lenght**	2-5 x 15-40 mm	1-2 x 10-25mm	5-20 x 40-70 mm	1-7 x 17-41 mm
	Leaf shapes	Density acute	Linear-oblanceolate to linear	Linear-oblanceolate to linear	Linear-oblanceolate to linear
	Leaf margins	*	Entire	*	Entire
	Leaf apices	*	Obtuse	*	Acute-Obtuse
	Shapes of the upper facial hairs	*	Lanate	*	Tomentose
	Shapes of the lower facial hairs	*	Lanate	*	Tomentose
	Leaf attachment	Subamplexicaul	Amplexicaul	Subamplexicaul	Subamplexicaul
	Colour	Grayish-green	Grayish-green	Yellowish-green	Yellowish-green
Flowers	**Flower shapes**	Capitulum	Capitulum	Capitulum	Capitulum
	State flowers of capitulum	From dense to lose corymbs	Dense corymbs	Dense corymbs	Lose corymbs
	Piece of flowers	*	5-9	*	4-9
	Length of involucre	4-9 mm	5-9 mm	4-9 mm	5-8 mm
Phyllaries	**Lenght**	*	1.5-2 x 2-3 mm	*	1-2 x 4-6 mm
	Shapes	*	Oblanceolate to Spatulate	*	Oblanceolate to Spatulate
	Base	Obtuse to Acute	Obtuse to Acute	Obtuse to Acute	Obtuse to Acute
	Margins	*	Entire	*	Entire
	Apices	*	Acute	*	Obtuse
	Shapes of the upper facial hairs	*	Pilose	*	Tomentose
	Shapes of the lower facial hairs	*	Pilose	*	Tomentose
	Colour	Yellow	Yellow	Yellowish-green	Yellowish-green
	Arrangement	Rarely imbricate	Loosely imbricate	Loosely imbricate	Loosely imbricate
Tubular flowers	**Colour**	*	Color from yellow to apricot	*	Apricot-colored
	Lenght	*	3-4 mm	*	3-3.5 mm
	Shapes of hairs	*	Tomentose	*	Tomentose

***:** Not any information about these features and characters in Flora of Turkey.

Anatomical properties:
***H. plicatum* subsp. *plicatum*:**
Root:

There is a single row of epidermal cells in cross-section taken from a radical formed. Under the epidermis is a layer of exodermis. Exodermis 4-5 layered, exodermis cells bigger the inner ones. Below are the cortex cells. Cambium is between the xylem and phloem. Cortex narrow from xylem structure. Tracheas is surrounded by sclerenchymatous environment. Cells of trachea and trakeid, sometimes arranged on a regular basis in secondary xylem. The pith consists of parenchymatic cells (Figure 5).

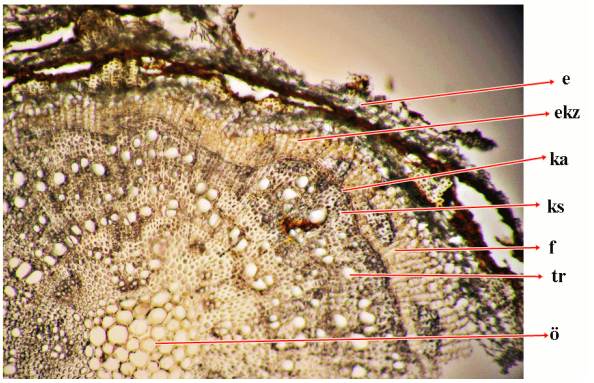


Figure 5. Cross-section of root of *H. plicatum* subsp. *plicatum*. e: epidermis, ekz: exodermis, ka: cambium, f: phloem, tr: trachea, ks: xylem, ö: pith

Stem:

The body on the cross-section contains cuticle layer of the epidermis is the outer. Often formed as a single cell array is located in the epidermis cells. There are eglandular hairs on the epidermis. Located under the cortex layer of the epidermis of a few. Cortex 2-4 layered. Transmission bundles arranged in a regular in stem. Sclerenchymatous cells thicken and they have narrow lumens. The pith consists of large parenchymatic cells. There is no pith cavity (Figure 6).

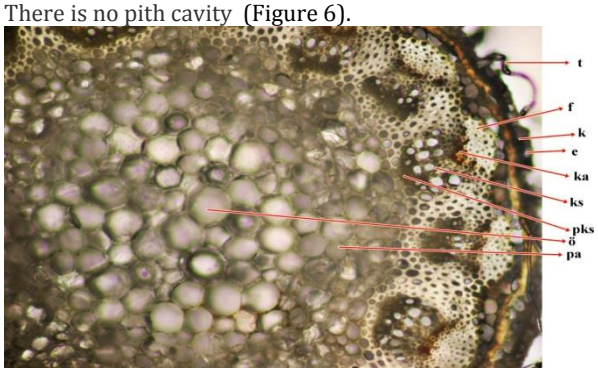


Figure 6. Cross-section of stem of *H. plicatum* subsp. *plicatum*. t: hair, e: epidermis, k: cortex, f: phloem, ka: cambium, ks: xylem, ö: pith, pks: primer xylem, pa: parenchyma

***H. plicatum* subsp. *polyphyllum*:**
Root:

The epidermis is composed of a single layer cells. There is 4-5 layered exodermis tissue below the epidermis. Below are the cortex cells. There is a cambium between phloem and xylem. Cambium cells are 2-3 layered. According to cortex is narrow structure to xylem. Phloem located in a restricted area according to xylem. Tracheas is surrounded by sclerenchymatous environment. Cells of trachea and trakeid, sometimes arranged on a regular basis in secondary xylem. The pith consists of parenchymatic cells (Figure 7).

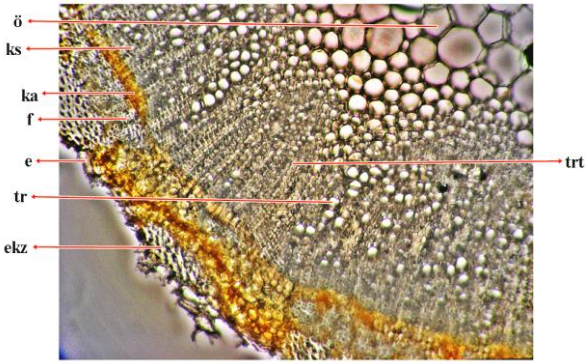


Figure 7. Cross-section of root of *H. plicatum* subsp. *polyphyllum*. e: epidermis, ekz: exodermis, ka: cambium, f: phloem, tr: trachea, ks: xylem, ö: pith, trt: trachead

Stem:

In cross-section, there is a cuticula layer in the outermost of stem. Epidermis is single layered and epidermal cells are arranged continuously. There are eglandular hairs on the epidermis. Transmission bundles arranged in a regular in stem. Cortex 1-2 layered. Cambium is distinguishable. Sclerenchymatous cells thicken and they have narrow lumens. The pith consists of large parenchymatic cells. There is no pith cavity (Figure 8).

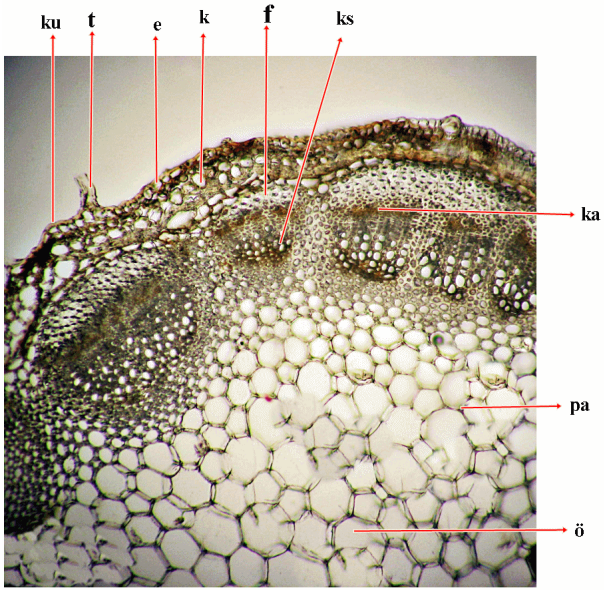


Figure 8. Cross-section of stem of *H. plicatum* subsp. *polyphyllum*. t: hair, ku: cuticle, e: epidermis, k: cortex, f: phloem, ka: cambium, ks: xylem, pa: parenchyma, ö: pith

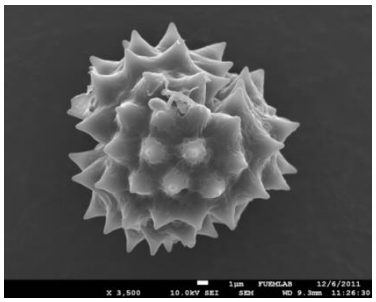
Palynological properties:

***H. plicatum* subsp. *plicatum*:**

Pollen grains are tricolporate, spheroidal. Polar view triangular, amb 14,83±1,16 µm. Polar axis 24,67±1,18 µm, equatorial diameter 24,67±1,29 µm, colpus length 13,77±2,10 µm, colpus width 3,11±1,02 µm, porus length 5,50±0,92 µm, porus width 4,50±0,92µm. Exine 2,2 µm. Spines are perforated (Figure 9).

***H. plicatum* subsp. *polyphyllum*:**

Pollen grains are tricolporate, oblate-spheroidal. Polar view triangular, amb 14,66±2,41 µm. Polar axis 21,90±1,56 µm, equatorial diameter 23,03±1,64 µm, colpus length 14,17±2,16 µm, colpus width 2,28±0,78 µm, porus length 5,14±0,94 µm, porus width 4,21±0,89 µm. Exine 2 µm. (Figure 10).



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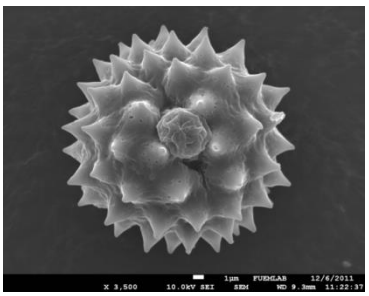
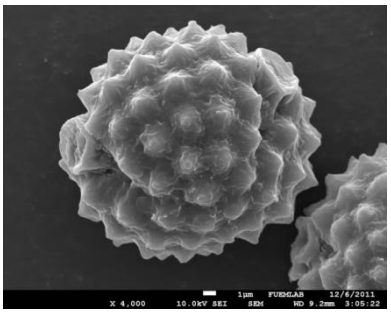


b

Figure 9. Scanning electron microscopy micrographs of pollen grains of *H. plicatum* subsp. *plicatum*. a. Polar view, b .Equatorial view. Scale bars; 1 µm.



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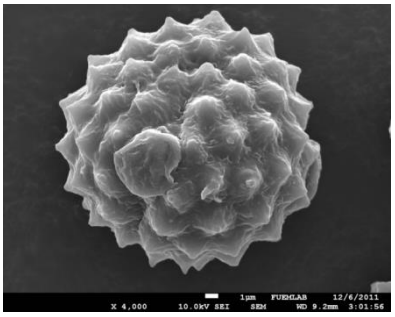

b

Figure 10. Scanning electron microscopy micrographs of pollen grains of *H. plicatum* subsp. *polyphyllum*. a. Polar view, b .Equatorial view. Scale bars; 1 µm.

4. Conclusion

In this study, detailed morphological characteristics, anatomical and pollen morphological structure of the *Helichrysum* taxa are given.

The determined morphological properties in our study are generally similar to the Flora of Turkey (Davis and Kupicha, 1975). But some different characters were reported in our study from Flora of Turkey (Davis and Kupicha, 1975). Such as basal leaves 1-2 x 10-13 mm, stem 4-42 cm tall, cauline leaves 1-2 x 10-25 mm, cauline leaves linear-oblanceolate to linear, cauline leaves attachment amplexicaul, state flowers of capitulum dense corymbs, length of involucre is 5-9 mm of *H. plicatum* subsp. *plicatum* in our study; however, it was reported in Flora of Turkey as basal leaves 2-10 x 10-100 mm, stem 4-42 cm tall, cauline leaves 2-5 x 15-40 mm, cauline leaves density acute, cauline leaves attachment subamplexicaul, state flowers of capitulum from dense to lose corymbs, length of involucre is 4-9 mm of *H. plicatum* subsp. *plicatum*. Moreover, basal leaves 1-2 x 10-45 mm, stem form erect, cauline 20-28 cm tall, stem hair condition tomentose, cauline leaves 1-7x17-41 mm, length of involucre is 5-8 mm, phyllaries loosely imbricate of *H. plicatum* subsp. *polyphyllum* in our study. However, basal leaves 2-10 x 10-100 mm, stem form erect or rarely oblique, cauline 4-42 cm tall, stem hair condition subglabrous to lanate-tomentose, cauline leaves 2-20x40-70 mm, length of involucre is 4-9 mm, phyllaries rarely loosely imbricate of *H. plicatum* subsp. *polyphyllum* in Flora of Turkey.

Both taxa were found different in throughout the plant, basal leaves leaf shapes, stem forms, state flowers of capitulum, tubular flowers colour and lenght features from each others. Morphological and morphometric characters of *H. plicatum* subsp. *plicatum* and *H. plicatum* subsp. *polyphyllum* findings are given in Table 2.

The anatomical investigations of *H. plicatum* subsp. *plicatum* and *H. plicatum* subsp. *polyphyllum* are reported for the first time in this study. The anatomy of the root and stem shows close similarities to the taxa studied and there was no different between taxa. The root and stem transverse sections were investigated. In transverse cross-sections of the upper root, it can be seen from the composition of the cortex structure during anatomic observations that secondary growth is a result of the plant's long-existence. This point has been stressed

in literature related to the subject (Metcalfe & Chalk 1972; Esau 1967; Fahn 1967; Ozorgucu et al. 1991; Ozorgucu 1993; Yentur 1995).

The pollen type of studied taxa were determined as tricolporate, *H. plicatum* subsp. *plicatum* pollen shape was spheroidal and *H. plicatum* subsp. *polyphyllum* pollen shape was oblate-spheroidal, echinate pollen ornemantation and showed differences in the size of pollen. However, another studies was reported that, *H. plicatum* subsp. *plicatum* and *H. plicatum* subsp. *polyphyllum* pollen shapes were oblate- spheroidal (Ok 2009; Inceoglu et al. 1977). In addition pollen porus width was 4,21±0,89 µm in our study of *H. plicatum* subsp. *polyphyllum*; Moreover, Ok (2009) was reported the same of with our study.

We believe that important discoveries unearthed during the study of morphological, morphometrical, anatomical and palynological features will lead to a better understanding of the taxa and provide a contribution to any future studies.

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