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A Morphological, Anatomical and Karyological Study on *Hieracium pannosum* BOISS. (Asteraceae)

Hakan Sepet[1]*, Bahattin Bozdağ[2] Canan Özdemir[3]

[1]* Kırşehir Ahi Evran University, Faculty of Engineering and Architecture, Department of Environmental Engineering, Kırşehir, Türkiye
[2]Menemen Anatolian High School Menemen-İzmir, Türkiye
[3]Manisa Celal Bayar University, Faculty of Arts and Sciences, Department of Biology, Manisa, Türkiye
* hakansepet@gmail.com
* Orcid No: 0000-0002-5841-9969

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Abstract

In this study Hieracium pannosum BOISS. morphological, anatomical and karyological features of the species were investigated. The samples were collected from Manisa Spil Mountain. The findings were compared with previous studies. We observed some changes in the morphological measurements of the species examined in the flora of Türkiye. In the anatomical studies, root stem and leaf characteristics of the species were determined. The body of the plant has a rounded shape in cross-section and contains 5-15 ordinary cortex layers. The body is self-parenchymatic. Palisate and sponge cells are similar in leaf mesophyll. The chromosome number of the species was determined as 2n = 27. Also, karyograms and idiograms were made.

Keywords: Anatomy, Hieracium pannosum, Karyology, Morphology.

1. Introduction

The Asteraceae family, one of the richest families on earth, is the second largest family in the Turkish Flora with 1156 species, and the geographical origin of this family is accepted as South America in phylogenetic terms [1-2]. *Hieracium* L. is in the Lactuceae tribe of the Asteraceae family and has more than 1000 species [3]. It is represented in Turkey with about 102 taxa [2-4]. This genus is distributed in mountainous areas all over the world [5]. Hieracium ssp. is found among the plants found in the pastures of Europe and North America [6-11]. *Hieracium* taxon has a wide variety of habitats. This genus has different taxonomic characters used in the classification of apomictic taxa. Due to the intense hybridization in the *Hieracium* genus, many difficulties are encountered in taxonomic classification [12-14].

In this study, the chromosome numbers of 8 Hieracium species collected from Turkey were studied. Different chromosome numbers ranging from 18 to 36 were determined for the species examined [15].

There is no anatomical and morphological study on the species *Hierecium pannosum*. The aim of this study is to determine the morphological, anatomical and karyological features of these species and contribute to their introduction.

2. Material and Method

2.1. Material

The plant samples in the study were collected from the following location where they naturally spread in Turkey.

B1 Manisa, Spil Mountain, At Alanı Location, 1520 m, 18.06.2012, Bozdag 055 (Figure 1).

Some of the plant samples obtained in the field studies were dried using standard herbarium techniques to be used in morphological studies. The dried plant samples are currently stored in the Celal Bayar University herbarium. Species identifications of the studied samples were made using fresh and dry samples using the 5th volume of Davis (1975)'s "Flora of Turkey".

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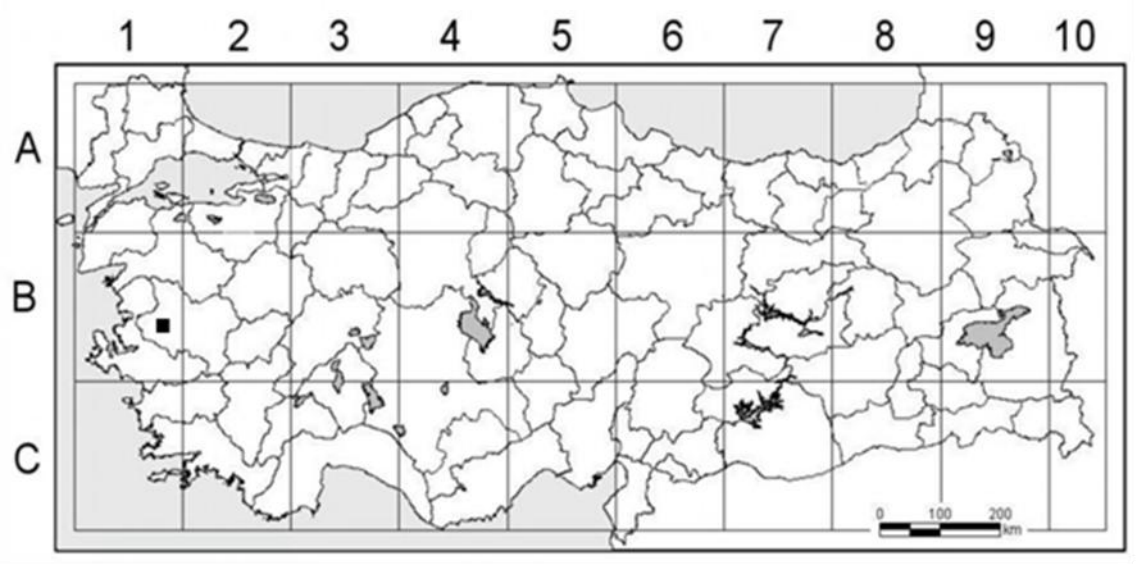

Figure 1. The Region Where the Species Examined Was Collected (Manisa, Spil Mountain)

2.2. Method

The parts taken from the roots, stems and leaves of the samples were stored in 70% alcohol. These samples were first passed through alcohol series in order. Then, the sections were taken from the paraffin blocks prepared with the parts taken from the roots, stems and leaves of this species for the studies [16] and these sections were stained with safranin and fast green dyes. Photographs were taken from the sections using a Leica DM3000 motorized microscope. The findings obtained are shown in figures and tables. The matured large seeds selected for karyological studies were left to germinate in petri dishes. 1-1.5 cm were cut from the root tips of the germinated seeds and exposed to the pretreatment agent (α-monobromonaphtalene) for 16 hours. The root tips removed from the pretreatment agent were hydrolyzed in 1 N HCl at 60 °C for 3-6 minutes. The hydrolyzed root tips were stained in Feulgen dye for 1.5 hours. After the stained root tips were washed several times with tap water, samples prepared with the crushing preparation method were examined for chromosome examination [17]. Photographs were taken from these preparations with a Leica DM3000 motorized microscope. The chromosome samples obtained from the photographs were named according to the method developed by Levan et al. (1964) [18].

3. Findings

3.1. Morphological Findings

The stem can grow up to 15-60 cm. The plant carries numerous stellate and long covering hairs. The leaves are 5-8(-12) in size, 40-200x20-55 mm. The leaves are denser in the lower part of the stem and can sometimes form a false rosette structure. The leaves are oblanceolate-oblong or obovate, usually obtuse, sometimes mucronate. They have entire or few small toothed structures. The leaves narrow towards the base and are attached to the stem more or less amplexally. The leaves are lanceolate with long plumose types. Capitula are 1-4 in number.

Peduncles extend up to 30 cm. They have 1-3 linear bracts. They have dense stellate and numerous long plumose hairs. The involucre is 13-20 mm in diameter. Phyllaries are narrowly linear-lanceolate, with a more or less acute apex and numerous stellate hairs. Phyllaries have small scattered yellowish glandular hairs, dense long plumose hairs. Ligules are globrous. Styles are yellow. Achenes are yellowish-brown and 3.5-4.5 mm in size. Flowering occurs in 6-9 months. It can usually be found among calcareous rocks, sometimes in stony areas or in open areas of forests. Its distribution is between 1000-2700 m (Figure 2).

Figure 2. A view from the natural habitat of the *H. pannosum* species.

3.2. Anatomical Findings

3.2.1 Root

At the outermost part of the root cross-section, there is a peridermis layer with fragmented cells and a cortex parenchyma consisting of 15-25 cell rows underneath. While the cell boundaries of these cells are clear in the young root cross-section, their walls are fragmented in the old root cross-section. In the young root, the endodermis layer immediately covering the vascular bundles is clear. In the root cross-section, the phloem cells have lost their integrity because their walls are fragmented. In the vascular tissue located in the central cylinder, the phloem layer covers a very small area. In contrast, xylem elements fill almost the entire center. In the old root of the plant, cambium cells can be seen here and there between the phloem and xylem elements (Figure 3, Table 1).

3.2.2 Stem

On the outermost part of the cross-section taken from the stem, there is a thin cuticle layer covered with a single row of epidermis cells, the widths of which are larger than their lengths. Just below the epidermis layer, there is a cortex parenchyma with cells that cover a very wide area and are larger than their lengths. In the vascular bundles, phloem cells are covered by sclerenchyma cells with thickened walls. Phloem cells cover a narrow area and their walls are fragmented. In the perennial stem, 1-2 rows of cambium cells can be distinguished between the vascular bundles. In the cross-section of the stem, the pith region covers a very wide area. The cells here are round-shaped and their diameters increase as they approach the center (Figure 4, Table 1).

3.2.3 Leaf

In the cross-section taken from the leaf, the cuticle width on the abaxial side is wider than on the adaxial side. There are two rows of epidermis cells on both sides of the leaf, the widths of which are larger than their lengths. The width and length of adaxial epidermis cells are larger than abaxial epidermis cells. There are stomata cells and dense covering hairs arranged at certain intervals on both surfaces of the leaf. The cells forming the mesophyll layer have shapes close to round. In the leaf mesophyll layer, the cells forming the palisade parenchyma and the cells forming the sponge parenchyma cannot be easily distinguished from each other. The width and length of epidermis cells are often larger than the dimensions of the cells forming the mesophyll layer. Sclerenchyma cells cover the phloem cells in the vascular bundles (Figure 5, Table 1).

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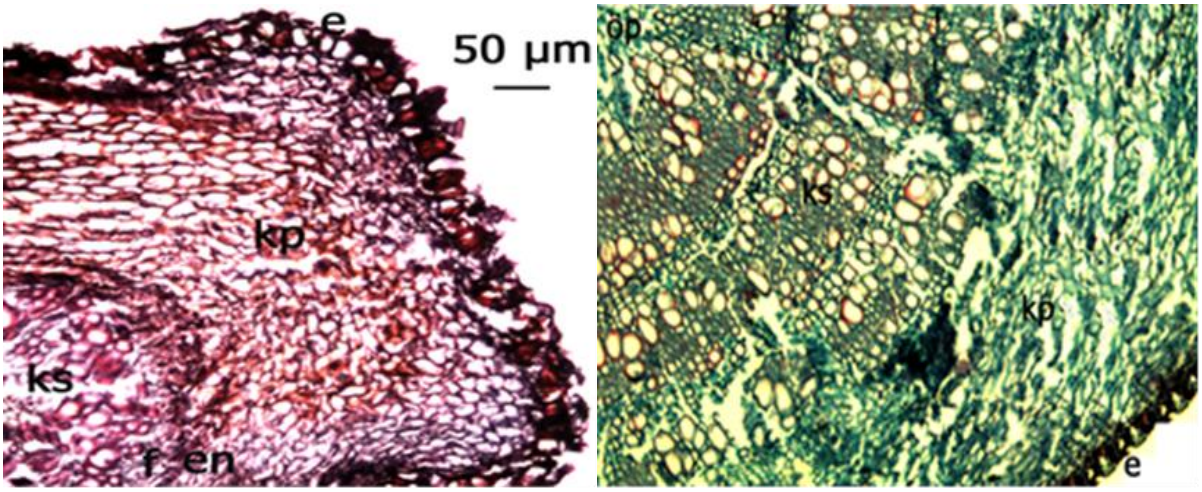

Figure 3. Anatomical sections of *H. pannosum* species A- young root, B- old root e. Epidermis end. Endodermis end. Peridermis end. Cortex parenchyma end. Xylem end. Sclerenchyma cells.

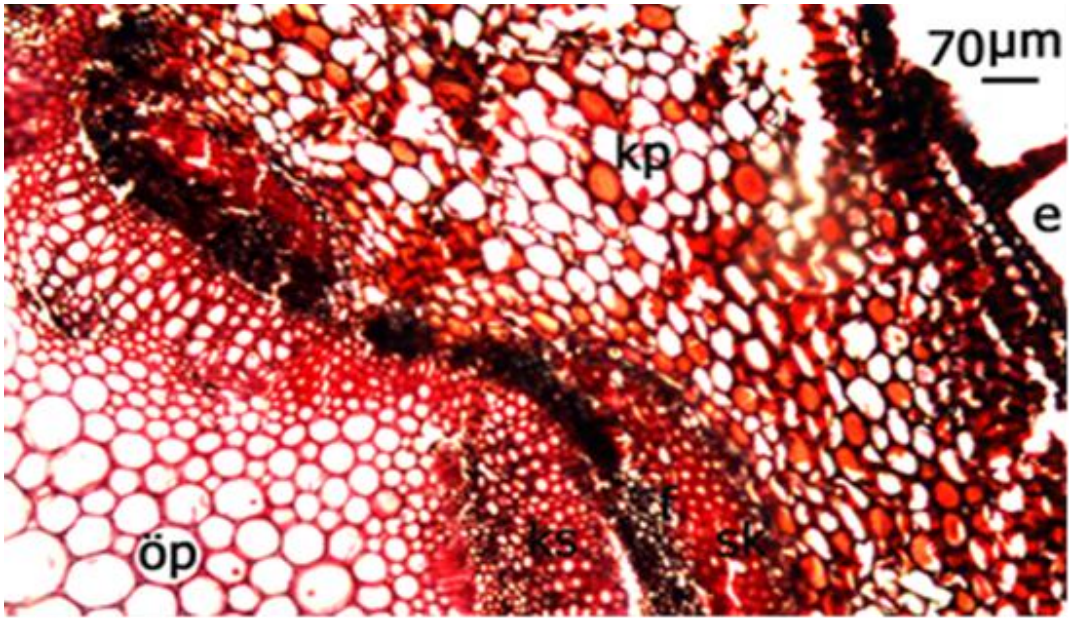

Figure 4. *H. pannosum* Stem cross-section, e. Epidermis, k. pith parenchyma, kp. Cortex parenchyma, sk. Sclerenchyma cells, f. Phloem, ks. Xylem.

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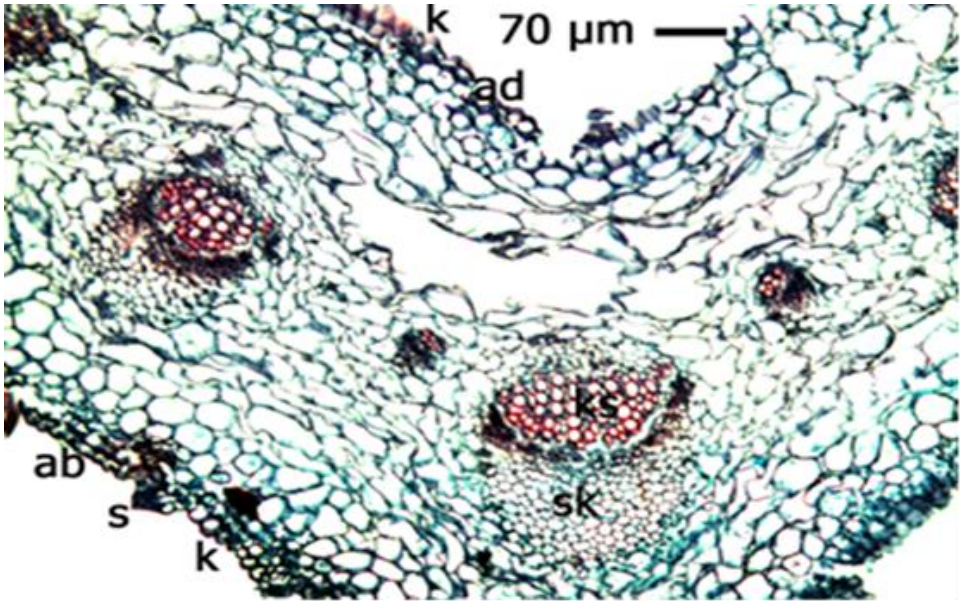

Figure 5. Leaf cross-section k. Cuticle, s. Stoma, ad. Adaxial epidermis, ab. Abaxial epidermis e. Epidermis, kiss. Pith parenchyma, ks. Xylem.

Table 1. Anatomical measurements of various parts of the species *H. Pannosum*

	width Min (µm)		**Max (µm)**	**Average**		**S.D*.**	**height Min (µm)**		**Max (µm)**	**Average**		**S.D*.**
Root												
Epidermis	12.25	-	40.00	24.13	±	8.88	16.25	-	30	22.63	±	5.19
Cortex parenchyma	30.00	-	47.50	37.25	±	5.97	11.25	-	22.50	16.63	±	3.54
Trachea (diameter)	15.00	-	40.00	28.50	±	9.29						
Stem												
Cuticle							2.50	-	6.25	3.50	±	1.29
Epidermis	10.00	-	20.00	15.88	±	3.01	11.25	-	22.50	17.75	±	3.57
Cortex parenchyma	25.00	-	62.50	40.25	±	13.87	15.00	-	43.75	30.75	±	12.06
Trachea (diameter)	12.50	-	30.00	21.00	±	5.03						
Spongy parenchyma	27.50	-	125.00	64.50	±	29.20						
Leaf												
Adaxial cuticle							1.25	-	5.00	2.94	±	1.38
Adaxial epidermis	16.25	-	32.50	21.88	±	4.97	15.00	-	25.00	20.50	±	3.96
Mesophyll cells	25.00	-	67.50	34.50	±	9.63	22.50	-	52.50	43.38	±	11.79
Trachea (diameter)	10.25	-	24.75	19.63	±	4.37						
Bundle sheath cells (diameter)	10.00	-	25.00	18.63	±	5.22						
Abaxial epidermis	12.50	-	21.25	18.13	±	3.19	12.50	-	22.50	15.38	±	2.70
Abaxial cuticle							1.88	-	5.00	3.25	±	1.13

S.D*. Standard deviation

3.3.Kariological Findings

Table 2. Measurements of mitotic metaphase chromosomes of *H. pannosum* species.

Chromosome no	Total chromosome length (C) µm	Long arm length (L) µm	Short arm length (S)µm	Arm ratio R: L/S	Centromeric index I: (S/C). 100 µm	Centromeric position S.D	Satellite
1	8.79	5.08	3.71	1.37	42.20	m	-
2	8.14	5.17	2.97	1.74	36.48	sm	-
3	7.86	4.90	2.96	1.65	37.66	m	-
4	7.68	4.62	3.06	1.51	39.84	m	-
5	7.12	4.80	2.32	2.06	32.58	sm	-
6	7.02	4.60	2.42	1.90	34.47	sm	-
7	6.94	4.24	2.70	1.57	38.90	m	-
8	6.48	4.44	2.04	2.17	31.48	sm	-
9	6.48	4.16	2.32	1.79	35.80	sm	-
10	6.48	3.88	2.60	1.49	40.12	m	-
11	6.22	4.07	2.15	1.89	34.56	sm	-
12	6.02	3.70	2.32	1.59	38.53	m	-
13	5.85	3.51	2.34	1.50	40.00	m	-
14	5.85	3.51	2.34	1.50	40.00	m	-
15	5.28	3.50	1.78	1.96	33.71	sm	-
16	5.28	3.43	1.85	1.85	35.03	sm	-
17	5.18	3.24	1.94	1.67	37.45	m	-
18	4.90	2.96	1.94	1.52	39.59	m	-
19	4.90	2.22	1.35	1.64	27.55	m	1.33
20	4.81	2.96	1.85	1.60	38.46	m	-
21	4.81	2.13	1.39	1.53	28.89	m	1.29
22	4.72	3.05	1.67	1.82	35.38	sm	-
23	4.63	2.77	1.86	1.49	40.17	m	-
24	4.35	2.59	1.76	1.47	40.46	m	-
25	4.26	2.59	1.67	1.55	39.20	m	-
26	4.16	2.59	1.57	1.65	37.74	m	-
27	3.79	2.31	1.48	1.56	39.05	m	-

The chromosome number of the species was determined as 2n=3x=27. In the karyotype analysis, submedian centromeres were determined in 9 chromosomes and median centromeres in 18 chromosomes. Satellites were observed in the 19th and 21st chromosomes. When the chromosomes of the species were examined, the largest chromosome was 8.79 µm and the smallest chromosome was 3.79 µm. The longest arm was 5.17 µm and the shortest arm was 1.35 µm. The total chromosome length was 158 µm. (Table 2, Figure 6, Figure 7)

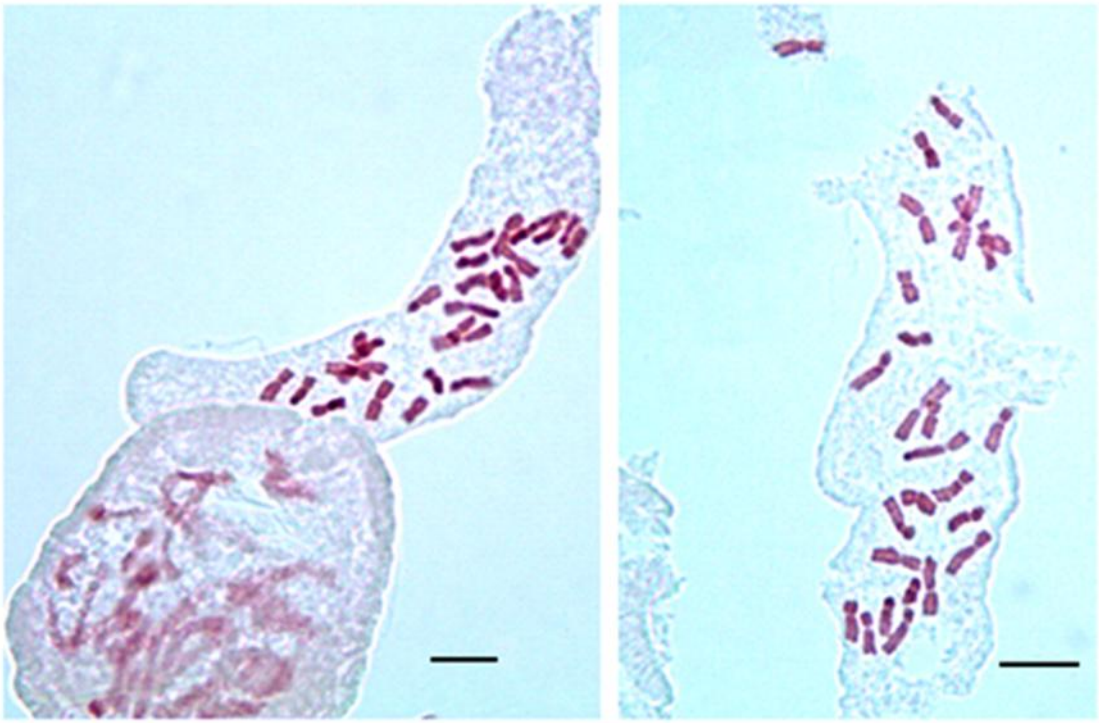

Figure 6. Somatic metaphase image of *H. pannosum* species (Scale 10µm)

Figure 7. Idiogram of *H. pannosum* species

4. Discussion and Conclusion

In this study, the anatomical, morphological and karyological structure of the *H. pannosum* species, which is included in the Asteraceae family, was examined and it was aimed to contribute to the introduction of the species.

There are dense covering hairs on the above-ground parts of the species. The phyllaries in the capitulum are in 4-7 rows and are arranged in an imbricate manner. The tips of the ligular flowers are indented and protruding in a sawtooth-like manner. The leaves are located at the base in the form of a rosette in most samples. The flower stalk also serves as a stem.

In anatomical examinations, the cortex parenchyma covers a very large area in the root of the species. Typical radial conduction tissue is evident in the young root. In the old root, xylem is evident in the conduction tissue and endodermis cells have disappeared. There is a wide pith region in the stem and the walls of the cells are thin and their diameters increase as they approach the conduction bundles. When old stems are examined, it is seen that xylem elements occupy a large space in the vascular bundles, phloem elements remain in a narrow area, and they are covered by sclerenchyma cells with thickened walls. It has been observed that the vascular bundles in the main vein of the leaves are larger. As we approach the leaf tips, it is seen that the vascular bundles are arranged at certain intervals. The length of the cuticle layer on the abaxial side of the leaf is larger than on the adaxial side. The width and length of the adaxial epidermis cells are larger than the width and length of the epidermis cells on the abaxial side. The distinction between palisade parenchyma and sponge parenchyma in the mesophyll layer is not clear. Mesophyll cells are generally round in shape.

The chromosome basic number in *Hierecium* species was determined as n = 9. There is also a lot of polyploidy in these species [15,19-20].

A study published in 2011 gave the chromosome numbers of *Hieracium* and *Pilosella* species distributed in Southeastern and Central Europe. In this study, the chromosome number of *H. pannosum* was determined as 2n=3x= 27 [21].

A study published in 2010 examined the *Hieracium* renatae species and stated that these species can live especially at altitudes of 2400-2520 m [22]. Materials belonging to the *H. pannosum* species were collected from an altitude of approximately 1250 m.

In a study conducted in Pakistan in 2009, it was stated that *Hieracium bichlorophyllum* (Druce & Zahn) Pugsley, *Hieracium diaphanoides* Lindeb., *Hieracium umbellatum* L., *Hieracium virosum* Pallas and *Hieracium vulgatum* Fr. species have dense hairy stems and bifacial leaves [6]. The H. pannosum species examined in this study also has dense hairy stems and leaves. The distinction between palisade parenchyma and sponge parenchyma in the leaves of the species is not clear. H. pannosum species has morphological, anatomical and karyological similarities to other Hieracium species. It is thought that morphological, anatomical and karyological studies will help in the scientific recognition of this species.

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