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ANATOMICAL AND MORPHOLOGICAL CHARACTERISTICS OF ENDEMIC *BARBAREA AURICULATA* TAXA (VAR. *AURICULATA* AND VAR. *PALUDOSA*)

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ABSTRACT. The aim of this study was to determine the anatomical and morphological characteristics of two endemic taxa of *Barbarea auriculata* naturally growing in Türkiye. In morphological studies, characteristics of the vegetative (root, stem, and leaf) and generative (flower, fruit, and seed) structures of both taxa were determined and detailed descriptions were given. Furthermore, according to the morphological data obtained, the identification key was revised again. In anatomical analyses, cross-sections were taken manually from the roots, stems, and leaves of the two taxa. The sections were made into permanent preparations and examined under a binocular light microscope. Sections were then photographed with an integrated camera system. The morphological and anatomical characteristics of both species are discussed in detail according to their similarities and differences.

1. INTRODUCTION

The family Brassicaceae is represented by 321 genera in the world [1] and 96 genera in Türkiye [2]. The genus *Barbarea* is represented by 29 species in the world [3] and 19 taxa belonging to 14 species in Türkiye, 11 of which are endemic [4,5]. Members of this genus are distributed in the warm regions of Eurasia, Australia, and North America, and in some countries of South America and the eastern parts of Africa.

Members of the genus *Barbarea* are biennial and perennial, rhizomatous or herbaceous plants with normal root structures, mostly distributed in humid environments or along riversides. Stems are erect, spreading, creeping, and sometimes recumbent. The basal leaves are simple, entire, lyrate, pinnatifid, or pinnatisect and the stem leaves are short-petiolate or sessile, auriculate, and enveloping (amplexicaul). *Barbarea* taxa are usually glabrous, but some have dense or sparse simple hairs on the stem and leaves. All taxa of the genus in Türkiye have yellow flowers and the inflorescence is racemose or paniculate.

Keywords. Anatomy, Barbarea, Brassicaceae, endemic, morphology.

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The fruit is silique, characteristic for the family. Seeds are in single rows, elliptic, ovate, brown, and swollen or slightly flattened.

Upon conducting a review of the relevant literature, it was concluded that there are no comprehensive studies of the anatomy of *Barbarea* taxa in either the world or in Türkiye to date. There are only a few limited studies of the taxa of this genus in Türkiye [6,7,8]. Therefore, in the present study, morphological and anatomical examinations of two *Barbarea auriculata* taxa (var. *auriculata* and var. *paludosa*) distributed in the Eastern Anatolia and Black Sea regions of Türkiye were carried out on specimens collected from different localities. All the data obtained are given in detail and discussed comparatively.

2. MATERIALS AND METHODS

2.1. Fieldwork and Collection of Samples

Field studies were carried out between 2006 and 2009 during the vegetation period from April to August. Samples for morphological studies were collected at both flowering and fruiting times of the taxa. While collecting samples, it was taken into account that they should be as numerous as possible to properly represent the respective populations. The collected specimens were pressed and dried according to general herbarium techniques [9] and prepared for identification. They were then identified using the relevant literature [10,11,12]. The specimens used in anatomical analyses were collected at maturity during field studies from the natural habitats of the taxa and preserved in 70% ethanol.

2.2. Morphological Studies

Morphological examinations were carried out primarily on specimens collected from field studies together with other specimens deposited in national herbariums (ANK, GAZI, EGE, HUB, and KNYA). All morphological and morphometric measurements were made on dry specimens and an average of at least 50 measurements were made for each taxonomic character to determine the limit of variation. Detailed descriptions were prepared according to the data obtained. Photographs of the morphological characteristics of the species were taken during field studies or in the laboratory.

2.3. Anatomical Studies

Anatomical examinations were carried out on specimens that were collected during field studies and placed in 70% ethyl alcohol. Anatomical sections of the root, stem, and leaf parts of the *Barbarea auriculata* taxa were prepared by hand. The sections were examined under a BX51 Olympus microscope with an integrated camera system and photographed in detail. The sections were organized using a computer, and tissue and cell parts were identified and shown in photographs. The specimens used in the anatomical studies and their localities are given in Table 1.

TABLE 1. Specimens used to anatomical studies and localities

Taxa	Collector No	Locality
Barbarea auriculata var. *auriculata*	Bağcı-3728	B7 Erzincan, Kemaliye, above Ergü village, stream and field banks, 29 June 2007, 1550-1600 m a.s.l
Barbarea auriculata var. *paludosa*	Bağcı-3698	B7 Erzincan, above Koçyatağı village, moist meadow areas, 26 June 2007, 2150 m a.s.l

3. RESULTS

In this section, the morphological and anatomical characteristics of endemic *Barbarea auriculata* taxa (var. *auriculata* and var. *paludosa*) distributed in Türkiye are given in detail. The root and stem anatomies of the taxa were evaluated separately, but since the leaf anatomies of the taxa are similar according to the data obtained, their leaf anatomical features are given together in the discussion section in detail.

3.1. Morphological Characteristics

3.1.1. *Barbarea auriculata* Hausskn. ex Bornm., in Mag. Bot. Lap. 30:55 (1931).

Perennial herb, usually with fibrous petiolar remains. Flowering stem erect, simple or branched, 15-55 cm, stems sometimes slightly hispid-setose or glabrous. Rosette leaves long petiolate, slightly retrorse-pilose, up to 14 cm, with orbicular to ovate terminal leaflets, 0.2-5.0 x 0.2-3.5 cm, slightly lobed or entire. Lower leaves with a large terminal leaflet and usually 0-1 pairs of lateral leaflets, sometimes 1-2 pairs of lateral leaflets, and distant, semi-amplexicaul auricles, larger than the lateral leaflets. Stem leaves petiolate or sessile, entire or repand-dentate to lobed, with large auricles. Upper stem leaves amplexicaul, crenate to dentate, sparsely pubescent, and sometimes glabrous. Inflorescence racemose or panicle, up to 20 cm, ebracteates or bracteates, if present similar to uppermost stem leaves. Nectaries 2, conspicuous. Flower buds glabrous or at least with one white simple hair at tip on some flowers. Sepals 3.5-4.5 x 2-2.5 mm, petals yellow, 6-8 x 2.0-2.5 mm, filaments 4-5.5 mm long, anthers 1.5-2.0 x 0.7-1.0 mm, pedicels 3-7 mm, slightly hispid or glabrous. Silique strict, torulose, glabrous, rarely slightly pubescent, linear at maturity, greenish or yellowish, valves conspicuously veined, 9-32 x 1.0-2.1 mm. Seeds in one row in each cell. Style (1.5-) 2-4 mm, stigma capitate, 2-lobed.

In the "Flora of Turkey", this species is given as two varieties according to whether the lower part of the stems are glabrous or retrorsely hispid-setose hairy. However, as a result of the examination of the specimens of both taxa, it was determined that both hairy and glabrous lower parts of the stems of both taxa

were present and it was understood that these characters could not be used in variety distinction. In this study, according to the new findings, pubescence of the fruit and stylus characteristics were used to distinguish the two taxa.

Diagnostic key to *Brabarea auriculata* taxa;

1. Stems glabrous below; fruit glabrous, stylus (2-) 2.5-3.5 (-4) mm …….. var. *auriculata*
1. Stems retrorsly hispid-setose below; fruit hairy, stylus 1.5-2.5 (-3) mm …… var. *paludosa*

3.1.1.1. *Barbarea auriculata* Hausskn. ex Bornm. var. ***auriculata***, in Mag. Bot. Lap. 30:55 (1931) (Figure 1).

The taxa is endemic to Türkiye and distributed only in the Eastern Anatolia region within the borders of the Kemaliye district of Erzincan province at 1500-600 m in fields and along stream banks (Figure 2). It usually blooms in April and May. The species is categorized as EN (Endangered) according to IUCN criteria. The endemic var. *auriculata* is known only from the type locality (Erzincan, Kemaliye) (Figure 2). It was not collected again after the year it was first described and was categorized as "Extinct in the wild-EX" in the Red Data Book of Turkish Plants published in 2000 [13]. However, it was collected again from the type locality both within the scope of this study (in 2007) and earlier by other botanists working in the region (in 2005) and was accordingly removed from the EX category.

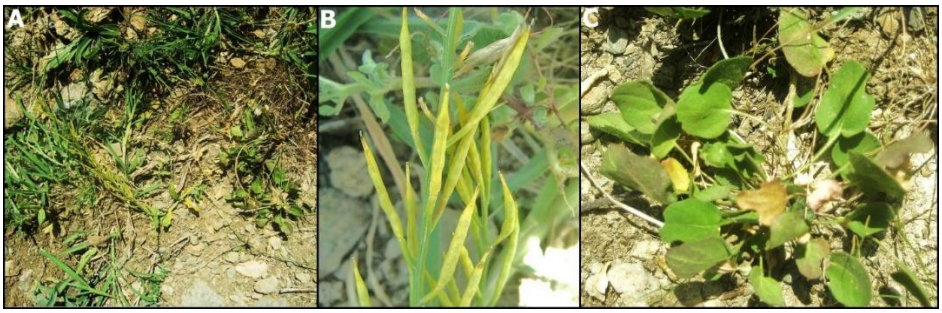

FIGURE 1. Morphological characteristics of *Barbarea auriculata* var. *auriculata*, A) habitus, B) fruit, C) rosette leaves

Distribution in Türkiye: B7 Erzincan: Kemaliye, above of Ergü village, stream and field edges, 29 June 2007, 1550-1600 m a.s.l. 39˚ 12' 828" N-38˚ 31' 428" E, *Bağcı-3728, Savran ve Başköse*; Kemaliye, above of Ergü village, 1580 m a.s.l., 10 April 2005, 39°12.59′N, 38°31.20′E, *Kandemir-6960*; Armenia Turcica: Egin (ad fluvium Euphratem) ad Argubaschi, Habenus Erek, 3 June 1890, *Sintenis-2460* (K!).

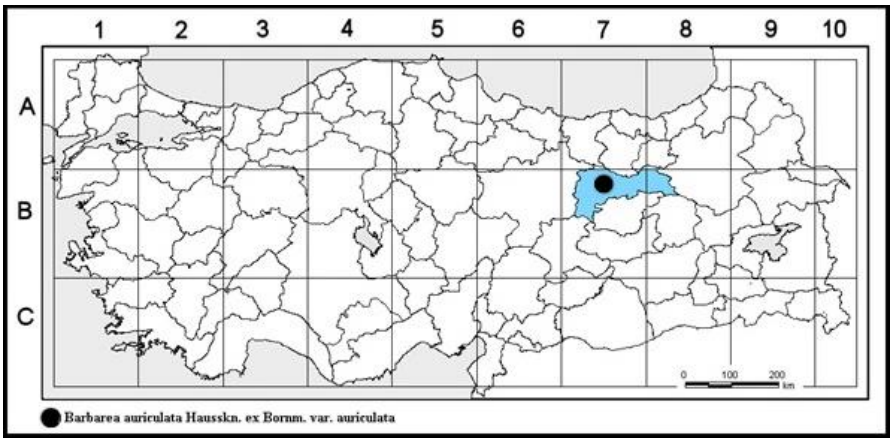

FIGURE 2. Distribution map of *Barbarea auriculata* var. *auriculata* in Türkiye

3.1.1.2. *Barbarea auriculata* Hausskn. ex Bornm. var. ***paludosa*** Coode & Cullen in Notes R.B.G. Edinburg. 26:000 (1964) (Figure 3 and 4).

FIGURE 3. Morphological characteristics of *B. auriculata var. paludosa,* A) inflorescence, B) flower, C-D) fruit, E) stem leaves, F) rosette leaves

The taxa is endemic to Türkiye and distributed in the provinces of Trabzon, Ardahan, Kars, Elazığ, and Erzincan in the Eastern Anatolia and Black Sea regions (Figure 5) between 1500 and 2650 m in moist meadows and wetlands

and along slopes and roadsides. The taxon usually blooms in May and June. It is categorized as VU (Vulnerable) according to IUCN criteria [13].

FIGURE 4. Habitus of *B. auriculata* var. *paludosa*

Distribution in Türkiye: A8 Trabzon: Between Trabzon and Gümüşhane, 2-3 km after the Zigana tunnel, to the right of the road, damp places, 1750 m a.s.l., 12 July 2009, 40˚ 38' 727" K – 39 22' 734" D, *Savran, Bağcı-4092*. **A9 Ardahan:**

Ardahan-Artvin road, 1-2 km before Çamlıbel pass (from Ardahan), roadside, 2600-2650 m a.s.l., 28 June 2007, 41˚ 12' 335" N - 42˚ 30' 469" E, *Bağcı-3714a, Savran*. **A9 Kars:** Arpaçay, Karakale Village, meadow, moist places, 1800-1900 m a.s.l., 27 June 2007, 40˚ 52' 000" N- 43˚ 27' 225" E, *Bağcı-3707, Savran*; Kars-Şavşat road, 15. km moist places, 2220 m a.s.l., 11 July 2009, 41˚ 11' 820" K – 42˚ 33' 171" D, *Bağcı-3979, Savran*, **B7 Elazığ:** Kuşakçı Mountain, around Yedipınar village, slopes and roadside, 1520-1780 m a.s.l., 21 May 2002, *Türkoğlu-3106*, **B7 Erzincan**: Above Koçyatağı village, moist meadow areas, 2150 m a.s.l., 26 June 2007, 39˚ 54' 483" N - 39˚ 13' 658" E, *Bağcı-3698, Savran*; Between Erzincan and Gümüşhane, Ahmediye Pass, east of BTV station, wetlands, 2100 m a.s.l., 20 June 2009, *Savran-3606*; Erzincan-Sivas Kızıldağ pass, eastern roadsides, wetlands, 2190 m a.s.l., 24 June 2009, *Savran-3680*.

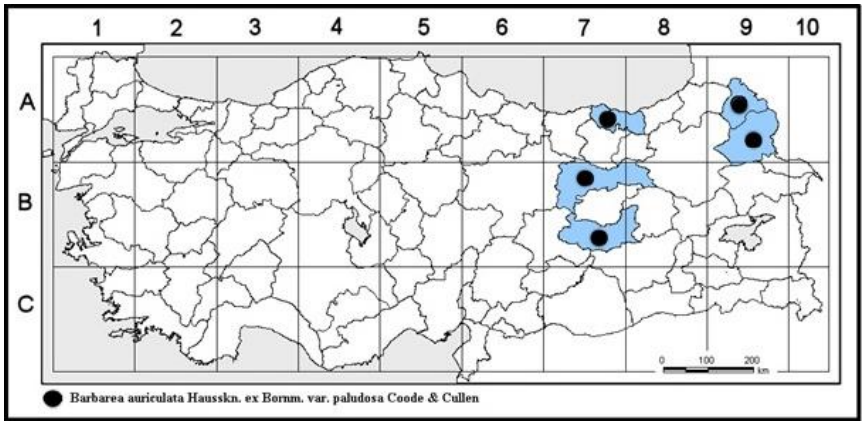

FIGURE 5. Distribution map of *B. auriculata* var. *paludosa* in Türkiye

3.2. Anatomical characteristics

3.2.1. *Barbarea auriculata* var. *auriculata*

Root Anatomy: Root is cylindrical. Periderm (exodermises) is multilayered (3-4) on the outer surface of the root (Figure 6B). Cortex tissue is very wide, 20- to 25-layered with parenchyma tissue under the periderm (Figure 6A). In the cortex tissue, cells store dense starch grains and are orbicular or oval-shaped (Figure 6B). There are also clustered sclerenchymatous cells in the parenchyma tissue in the cortex. There are no significant lines between the pith region and the cortex. The cambium is formed between the phloem and xylem in the order of 2-3 cells. In addition, the presence of three separate rings in this taxon indicates that the plant is perennial. The xylem was found as concentric rings below the cambium (Figure 6C). The cambium periodically produces lignified and unlignified tissues. Sclerenchyma cells were seen in the lignified xylem area. The

sclerenchyma tissue fills intensely between xylem tissues. Parenchyma tissue is also observed in the pith region (Figure 6D).

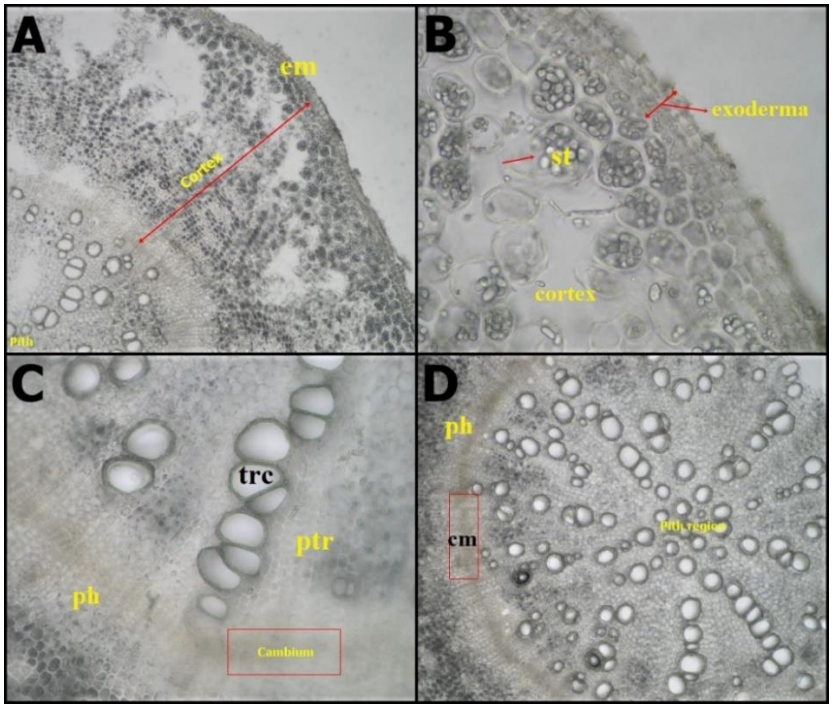

FIGURE 6. Root Cross-section of *Barbarea auriculata* var. *auriculata*, A) cortex and pith region (10X), B) cortex and exoderma (40X), C) trachea, pith rays and cambium (40X), D) pith region (10X), (cm: cambium, em: exoderma, ph: phloem, ptr: pith ray, st: starch, trc: trachea)

Stem Anatomy: It can be understood that the stem structure is not cylindrical but canaliculated (grooved) due to the outward projections (Figures 7A, 7D). In transverse section, the outermost part of the stem has a single layer of epidermis with a thick cuticle (Figure 7B). In some sections, the epidermis appears to be 2-rowed, in which case the second lower layer is called the collenchyma (Figure 7C). Stomata of the amphistomatic type are present on the epidermis. The cortex layer consists of parenchyma in 5-6 layers, thin-walled, with regularly oval or rounded cells. The outer parenchyma cells of the cortex contain chloroplast, while inner cortex cells contain dense starch granules. Below the cortical parenchyma is the endodermis, which consists of a single layer of orbicular or oval-shaped cells. There are no starch granules in the endodermic cells. In vascular bundles, the phloem consists of 4-5 cell layers (Figures 7E, 7F) and there is also lunar-shaped sclerenchyma tissue with 1-2 cell rows on the outer part of the phloem (Figures 7D, 7E). Tracheid cells are especially evident in the area of the xylem. The cambium is distinguished between the xylem and phloem

tissues. The sclerenchymatous tissue is located between vascular bundles, in 5-6 layers and consisting of irregular and squashed cells (Figure 7G). Cambium cells are not clear (Figure 7E). The pith region occurs in the center of the stem, composed of large orbicular or polygonal parenchymatic cells (Figures 7H, 7I). In the anatomical structure of mature stems, these cells fragment in the center to form rexigenous spaces (Figure 7H), but this is not seen in young stems (Figure 7I).

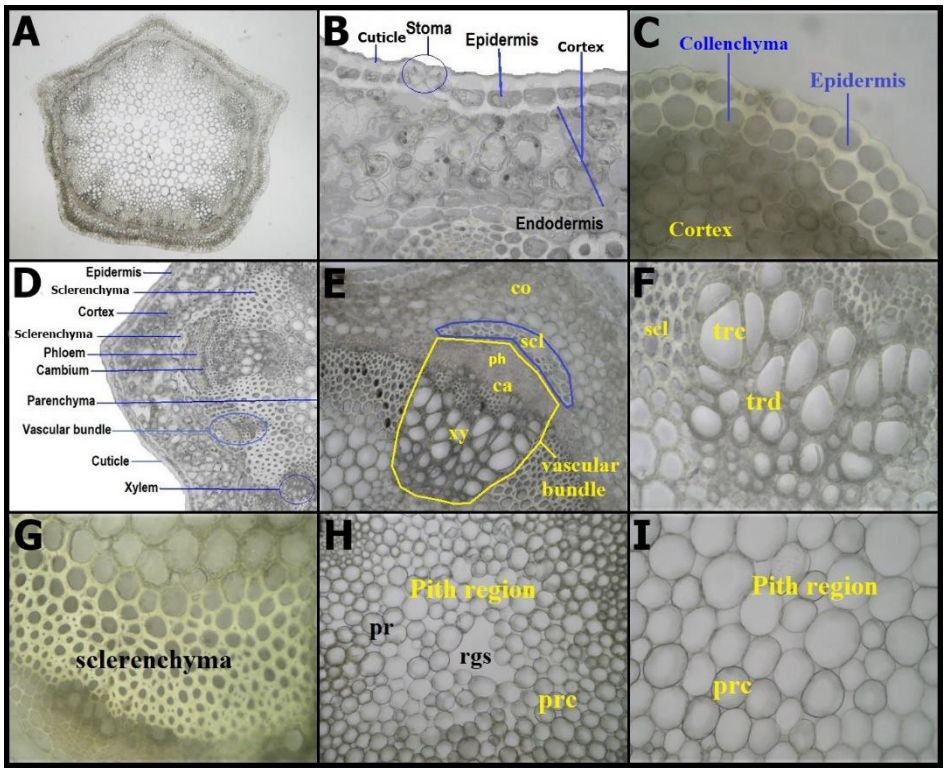

FIGURE 7. Stem Cross-section of *Barbarea auriculata* var. *auriculata*, A) general stem section (4X), B) epidermis and cortex (20X), C) epidermis and collenchyma (20X), D) general anatomic parts (10X), E) vascular bundle (20X), F) trachea and tracheid (40X), G) sclerenchyma tissue (40X), H) pith region (adult plant, 20X), I) pith region (young plant, 40X). (ca: cambium, co: cortex, fl: phloem, pr: parenchyma, prc: parenchymatic cells rgs: rexigenous spaces, scl: sclerenchyma, trc: trachea, trd: tracheid xy: xylem)

3.2.2. *Barbarea auriculata* var. *paludosa*

Root Anatomy: Root is cylindrical. Exodermis is multilayered (3-4) on the outer surface of the root. A secondary structure was seen, far from the periderm, with the cortex layer below. Cortex tissue is very wide, 15- to 20-layered, with parenchyma tissue under the periderm. In the cortex, tissue cells store dense

starch grains and are orbicular or oval-shaped. There are also clustered sclerenchymatous cells in the parenchyma tissue in the cortex. There are no significant lines between the pith region and the cortex. The cambium is formed between the phloem and xylem in the order of 3-4 cells. The presence of three separate rings in this taxon indicates that the plant is perennial. The xylem was found as concentric rings below the cambium, which periodically produces lignified and unlignified tissues. Sclerenchyma cells were seen in the lignified xylem area. The sclerenchyma tissue fills intensely between xylem tissues. Parenchyma tissue is also observed in the pith region (Figures 8A-8D).

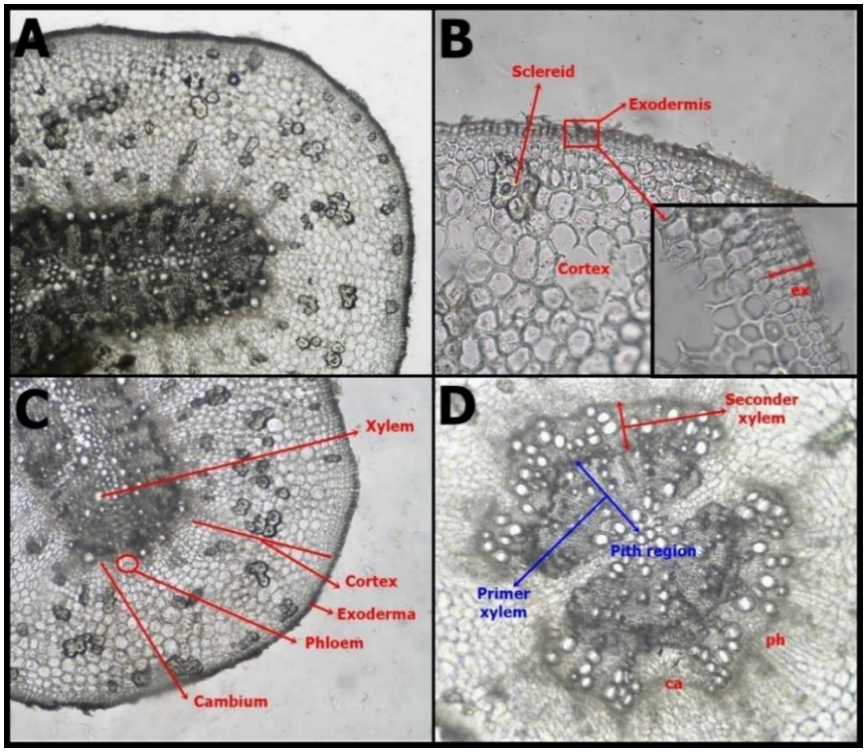

FIGURE 8. Root Cross-section of *Barbarea auriculata* var. *paludosa* A) general stem section, B) exodermis and cortex, C) general anatomic parts of root, D) pith region and vascular bundle, (ca. cambium, ex: exodermis, ph: phloem)

Stem Anatomy: It can be seen from the stem cross-section that the body structure is not cylindrical (Figure 9A). In transverse section, the single-layered epidermis was found with thick cuticle on the outermost part of the stem. The cells of the epidermis are cubic (Figure 9B). Stomata of the amphistomatic type are present on the epidermis. Cortex layer consists of parenchyma in 3-5 layers, thin-walled, with regularly oval or round cells. The outer parenchyma cells of the cortex contain chloroplast, while inner cortex cells contain dense starch granules. Underneath the cortex parenchyma, there is a single-layered

endodermis with cells orbicular or oval-shaped. The endodermal cells store starch granules. In vascular bundles, the phloem consists of 4-5 cell layers and xylem tissue externally surrounds it. Tracheid cells are especially evident in the area of the xylem. The cambium is distinguished between the xylem and phloem tissues (Figures 9E, 9F). Cambium cells were not clear. Sclerenchymatous tissues is located between vascular bundles (Figures 9C, 9D) in 5-6 layers and consists of irregular and squashed cells (Figure 9G). There is also lunar-shaped sclerenchyma tissue with 1-2 cell rows on the outer part of the phloem (Figure 9E). In the center of the stem is the pith region, composed of large orbicular or polygonal parenchymatic cells (Figures 9H, 9I). These cells may disintegrate in the center to form rexigenous spaces (Figure 9H). Sometimes, in mature stems, the central part may be completely hollow (Figure 9I).

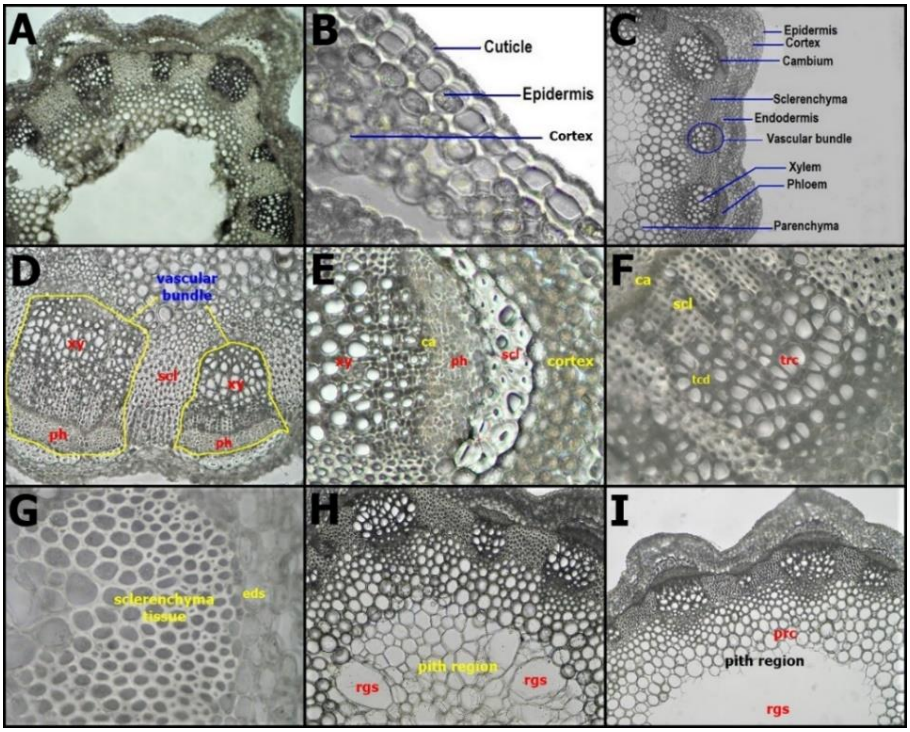

FIGURE 9. Stem Cross-section of *Barbarea auriculata* var. *paludosa* A) general stem section (4X), B) epidermis and cortex (40X), C) general anatomic parts (10X), D) vascular bundle (20X), E) vascular bundle parts (40X), F) trachea and tracheid (40X), G) sclerenchyma tissue (40X), H) pith region (young plant, 20X) I) pith region (adult plant, 20X). (ca: cambium, eds: endodermis, ph: phloem, pr: parenchyma, prc: parenchymatic cells, rgs: rexigenous spaces, scl: sclerenchyma, trc: trachea, tcd: tracheid xy: xylem)

4. DISCUSSION

4.1. Morphology

The geographical distribution areas and altitudes of these taxa are different. The only common distribution area occurs in Erzincan province (Figure 2 and Figure 5). *Barbarea auriculata* var. *paludosa* has a wider distribution area and is distributed at higher altitudes than var. *auriculata* (Figure 2 and Figure 5). Var. *auriculata* is known only from its type locality, the Kemaliye district of Erzincan province, and is categorized as CR (Critically Endangered) according to IUCN criteria [13].

Although these taxa of *Barbarea auriculata* (var. *auriculata* and var. *paludosa*) are morphologically similar, they differ in stem length, terminal leaflet structure of the rosette leaves (Figures 10A, 10C), and fruit and style size (Figures 10B, 10D). Comparative differences between the taxa are given in Table 2.

TABLE 2. Morphological differences between the two varieties

Taxa► Characters▼	***Barbarea auriculata* var. *auriculata***	***Barbarea auriculata* var. *paludosa***
Stem lenght	18-43 cm	15-55 cm
Rosette leaves length	up to 12 cm	up to 14 cm
Terminal leaflets size	0.2-3.5 x 0.2-2.3 mm	0.7-5.0 x 0.5-3.5 cm
Silique hair type	usually glabrous, rarely slightly pubescent	usually pubescent, sometimes glabrous
Silique size	9-26 x 1.0-2.06 mm	15-32 x 0.5-1.7 mm
Style size	(2-) 2.5-3.5 (-4) mm	1.5-2.5 (-3) mm

4.2 Anatomy

4.2.1 Root Anatomy

In anatomical studies, it was determined that although the two taxa are similar in their root, stem, and leaf anatomies, there are some differences between them.

Based on root anatomy data, it was determined that the root anatomies of the taxa were similar. On the other hand, the existence of some anatomical differences was observed in detail. The most important difference is the presence of a secondary structure in the root of *Barbarea auriculata* var. *paludosa* (Figure 8D). There are therefore both primary and secondary xylem elements in the central cylinder of the root of this taxon. In var. *auriculata*, there are only primary xylem elements in the pith region due to the presence of the primary structure (Figure 6D). The second important difference is the number of layers of parenchymatic cells forming the cortex. In var. *auriculata*, the cortex consists of 20-25 layers of parenchyma cells (Figure 6A), while in var. *paludosa* it consists of 15-20 cell layers (Figures 8A, 8C). Third, there is a difference in the number

of cell layers of the cambium between the xylem and phloem. In var. *auriculata* the cambium consists of 2-3 cell layers (Figures 6C, 6D), whereas in var. *paludosa* it consists of 3-4 cell layers (Figure 8D).

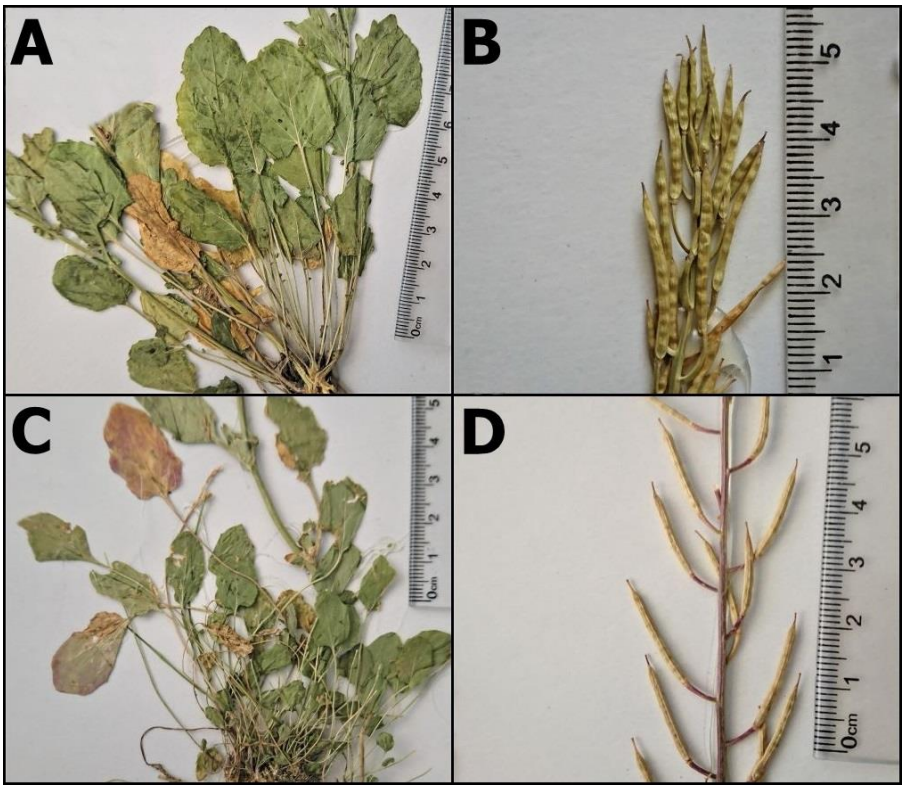

FIGURE 10. Morphological differences between the taxa, A-B) *Barbarea auriculata* var. *auriculata*, C-D) *Barbarea auriculata* var. *paludosa*, (A and C rosette leaves, B and D fruit structures)

Sclereids are found in dense, scattered, or small clusters among the parenchyma cells in the cortex layer of var. *paludosa*. This is rarely seen in var. *auriculata*. The presence of supportive tissue elements such as sclerenchyma in the root cortex structure of many plants is consistent with the literature data [14].

4.2.2 Stem Anatomy

Although the stem anatomical structures of the taxa are similar, some structural differences were also observed. The most important difference is in the epidermis layer. Both uni-layered and bi-layered epidermis can be observed in var. *auriculata*. In the latter case, this second layer is called a collenchyma (Figures

7B, 7C). In contrast, in var. *paludosa*, the epidermis consists of only a single layer of densely arranged cubic or round cells (Figure 9B).

Another difference is the number of parenchymatic cell layers of the cortex. In var. *auriculata*, the parenchyma cells of the cortex consist of 5-6 cell layers (Figures 7B-7D), while in var. *paludosa* this number is 3-5 (Figures 9B, 9C). In plants, the width of the cortex layer and the number of cell layers vary from species to species and according to the developmental conditions of the plant [14].

According to the literature, starch can be found intensively in the endodermis cells of many species [15]. However, this was not observed in the endodermis cells of these two *Barbarea auriculata* varieties.

In addition, sclerenchyma tissue is also densely located between vascular bundles in both taxa. This has also been observed in other taxa of the family [16].

4.2.3. Leaf Anatomy

Since the studied varieties belong to the same species, the anatomical study revealed that their leaf anatomical structures and characteristics were the same. This is true for the majority of the other taxa of the genus *Barbarea*. For this reason, the leaf anatomy of both varieties is described here for a single specimen.

When the leaf anatomy was examined, it was determined that the leaves are of the type referred to as bifacial or dorsiventral. In this type, the leaf mesophyll layer consists of two separate parenchyma tissues, the palisade layer and sponge layer, and it is the most common type among species in nature. Although anatomical studies have shown that leaf anatomy differs between taxa of both Brassicaceae and other families [14,16–19], the mesophyll structures of the two considered varieties were found to be similar in this study.

Although the cells forming the upper and lower epidermis layer are densely arranged, they differ in cell size (large or small) and shape (round, cubic, or oval). There is a thin layer of cuticle over the epidermis layer. There are stomata between the epidermis cells (upper or lower). The palisade parenchyma is located under the upper epidermis layer and consists of 2-3 layers with densely arranged long cylindrical cells with abundant chloroplasts. The sponge parenchyma is located above the lower epidermis layer and consists of 4- to 5-layered, scattered, differently shaped cells with few chloroplasts and intercellular spaces. There are conduction bundles at the junction of both tissues. The general leaf anatomy and parts are detailed in the figure below (Figure 11).

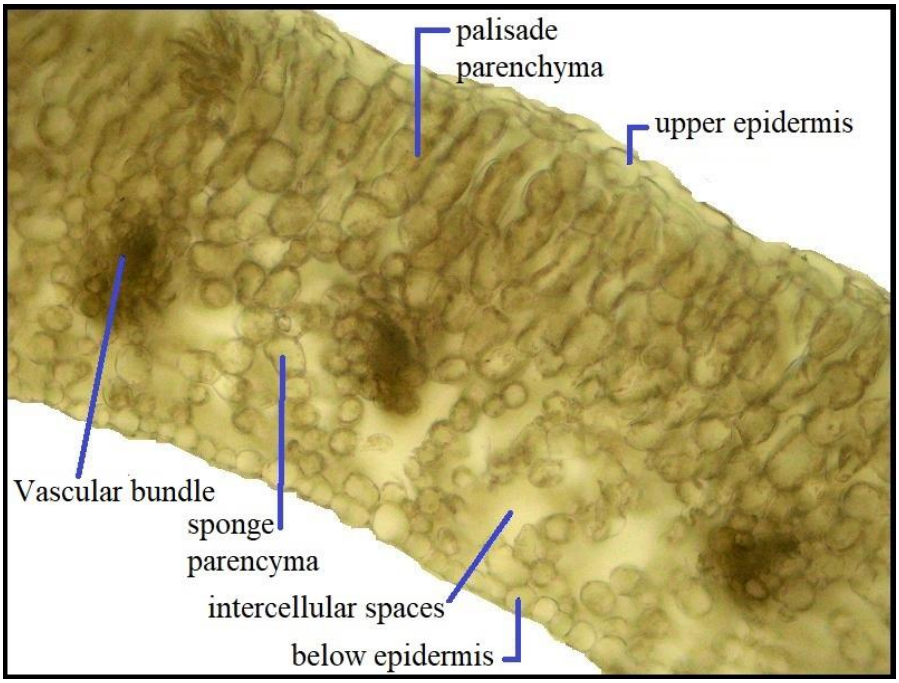

FIGURE 11. *Barbarea* leaf cross-section, anatomical parts, (magnification 20X)

5. CONCLUSIONS

In this study, the morphological characteristics of the taxa *Barbarea auriculata* var. a*uriculata* and *Barbarea auriculata* var. *paludosa* were determined in detail. Base on the data obtained, the differences between the two taxa were discussed and the diagnostic key was revised to identify the taxa. In addition, the comprehensive anatomy of endemic varieties of *Barbarea auriculata* was determined for the first time in this study. Anatomical differences between the two taxa are discussed.

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