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ANATOMICAL AND POLLEN CHARACTERS IN THE GENUS *EPILOBIUM* L. (ONAGRACEAE) FROM NORTHEAST ANATOLIA

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Anatomical and palynological features of *E. algidum* Bieb., *E. palustre* L., *E. ponticum* Hausskn., *E. confusum* Hausskn., *E. hirsutum* L. and *E. montanum* L. collected from NE Anatolia were examined and evaluated by numerical analysis in order to determine the taxonomic value of the observed internal peculiarities. Features related to pollen shape and ornamentation, idioblast distribution, number of palisade parenchyma rows and the presence and distribution of sclerenchyma fibers were found to be important in separating the examined taxa. Principal component analysis showed that the anatomical characters are more important than the palynological ones in explaining the total variation among the examined taxa.

Key words: *Epilobium*, numerical analysis, anatomical characters, pollen, Turkey.

INTRODUCTION

The genus *Epilobium* L. (Onagraceae), with about 185 species throughout the world (Raven, 1976), is a very difficult group taxonomically because of its fairly uniform external appearance and the high possibility of hybridization among almost all species (Akbari, and Azizian, 2006). In addition to phenetic characteristics, seed and pollen features have been employed as important characters within the genus (Akbari, and Azizian, 2006). In recent years the relationships among *Epilobium* species have been explored in various studies, most of them focused on pollen features and seed morphology; there has been very little study of anatomical properties. Metcalfe and Chalk (1950) reported the general anatomical properties of Onagraceae, including a few details on the genus *Epilobium*. Pollen grains of Onagraceae received much attention in early work (Erdtman, 1952), but these studies considered only brief stages. Recent palynological studies on *Epilobium* focus mainly on the development of microspores in mature pollen grains (Keri and Zetter, 1992), and exine structure (Rowley and Claugher, 1996).

The genus *Epilobium* is represented by 21 species in Turkey, growing mostly in humid open grassland and on roadsides (Chamberlain and Raven, 1972). According to Chamberlain and Raven (1972), most of the Turkish representatives are poorly defined and require additional characters for certain identification. Some Anatolian species (aerial parts or roots) are used in folk medicine against prostate and gastrointestinal disorders (Zeybek and Zeybek, 1994). The present study aims to examine the anatomical and palynological properties of NE Anatolian representatives of *Epilobium* and to evaluate their discriminative potential as taxonomic characters.

MATERIAL AND METHODS

PLANT MATERIAL

Plants were collected from Rize and Trabzon (Turkey) in 2004–2005 (Fig. 1). The collection data for the examined specimens are given in Table 1. Specimens were dried according to standard herbarium techniques and are stored in the Herbarium of Karadeniz Technical University, Department of Biology (KTUB).

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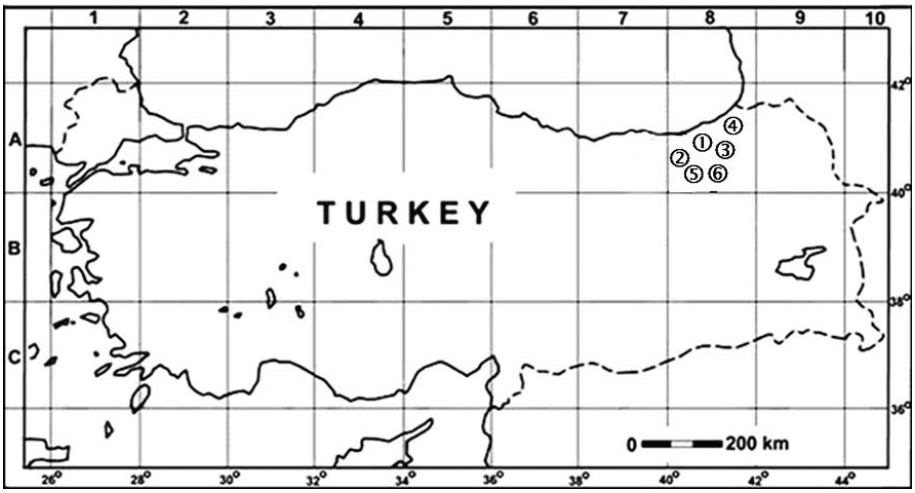


Fig. 1. Distribution map of the investigated taxa. 1 – *E. montanum*; 2 – *E. hirsutum*; 3 – *E. algidum*; 4 – *E. palustre*; 5 – *E. ponticum*; 6 – *E. confusum*.

TABLE 1. Localities of the examined *Epilobium* taxa

Taxa	Locality
E. algidum Bieb.	A8 Rize: Cimil, 1800 m, 11.07.2005, Makbul 100, KTUB
E. palustre L.	A8 Trabzon: Camiboğazı, 1650 m, 05.08.2005, Makbul 101, KTUB
E. ponticum Hausskn.	A8 Rize: Cimil, 1960 m, 15.07.2005, Makbul 108, KTUB
E. confusum Hausskn.	A8 Rize: Cimil, 2000 m, 15.07.2005, Makbul 107, KTUB
E. hirsutum L.	A8 Trabzon: Uzungöl, 1600 m, 20.07.2005, Makbul 106, KTUB
E. montanum L.	A8 Trabzon: Camiboğazı, 1800 m, 05.08.2005, Makbul 102, KTUB

ANATOMICAL STUDIES

The materials for anatomical study were fixed in FAA (formaldehyde:acetic acid:alcohol) for 24 h and then preserved in 70% alcohol in the field. Transverse sections of stems and leaves, and upper and lower leaf epidermises excised by hand were examined. All sections were stained with safranin-fast green for 24 h and mounted with glycerine-gelatine to make permanent slides (Vardar, 1987). Well-staining sections were photographed with an Olympus BX51 from permanent slides. All measurements and observations were made three or four times from several sections taken from at least two selected specimens.

PALYNOLOGICAL STUDIES

Pollen was obtained from herbarium specimens. The pollen slides were prepared according to Wodehouse (1935). For LM, pollen grains were dissected from herbarium specimens and placed on clean microscope slides, and 2 or 3 drops of 96% ethanol were added to melt the resin and oil. Glycerin-gel with basic fuchsin added was placed on the pollen and allowed to melt and mixed with a clean pin to scatter the pollen grains. The pollen grains were photographed with an Olympus BX51 from permanent slides. Size measurements were averaged from 30 pollen grains.

The ornamentation of these specimens was determined (Punt et al., 1994). For SEM, unacetolyzed pollen grains were put on stubs, coated with gold (SC502 Sputter Coater) and photographed with a JEOL JSM–6060 LV scanning electron microscope.

NUMERICAL ANALYSIS

As summarized in Table 2, 43 characters were assessed by numerical analysis: 27 related to anatomical and 16 related to palynological features. These include quantitative and binary variables. Arithmetic means of each quantitative variable related to anatomical properties and pollen grains were calculated for each taxon separately in order to determine the values of particular characters for each taxon. Two multivariate analyses were performed using SYN-TAX PC 5.0 (Podani, 1993): cluster analysis (CA) and principal component analysis (PCA). For CA, a pair-wise matrix of resemblance values was calculated from the raw standardized data matrix, using Gower's coefficient of resemblance for mixed data sets (Sneath and Sokal, 1973). For PCA, the raw data were used to create a correlation matrix, and two eigenvectors were

TABLE 2. Characters used in this study

Symbol	Character
X_1	Width/length of epidermal cells of stem (µm/µm)
X_2	Width/length of collenchyma cells of stem (µm/µm)
X_3	Width of cortex/ Width of cylinder (µm/µm)
X_4	Width of cortex (µm)
X_5	Average number of cortex cells (mm²)
X_6	Width of pith (µm)
X_7	Average number of trachea (mm²)
X_8	Width of xylem (µm)
X_9	Width of phloem (µm)
X_{10}	Scleranchyma fibers on phloem; present:1, absent:0
X_{11}	Width/length of endodermal cells of stem (µm/µm)
X_{12}	Average number of pith cells (mm²)
X_{13}	Number of series of palisade parenchyma
X_{14}	Width of palisade parenchyma (µm)
X_{15}	Width/length of upper epidermal cells
X_{16}	Average number of upper epidermal cells (mm²)
X_{17}	Width/length of lower epidermal cells
X_{18}	Width/length of lower epidermal stomata
X_{19}	Average number of lower epidermal cells (mm²)
X_{20}	Average number of lower epidermal stomata (mm²)
X_{21}	Stomata index of lower epidermis
X_{22}	Width of palisade tissue / Width of spongy tissue
X_{23}	Leaf amphistomatic:1, epistomatic: 0
X_{24}	Length of raphid crystal bundle
X_{25}	Width/length of leaf vascular bundle
X_{26}	Width/length of upper epidermal cells/ Width/length of lower epidermal cells
X_{27}	Average number of raphid crystal bundle (mm²)
X_{28}	Amb (µm)
X_{29}	Apoporium (µm)
X_{30}	se: Sexine (µm)
X_{31}	Pollen shape: oblate, suboblate: 0 sphaeroidea:1
X_{32}	E: Equatorial axis (µm)
X_{33}	P: Polar axis (µm)
X_{34}	e: Exine at top of pore canal (µm)
X_{35}	plg: Length of pore (µm)
X_{36}	plt: Width of pore (µm)
X_{37}	in: Intine (µm)
X_{38}	t: Top of pore canal (µm)
X_{39}	ne: Nexine (µm)
X_{40}	P/E rate
X_{41}	m: Mesoporium (µm)
X_{42}	c: Width of costae (µm)
X_{43}	ex: Exine (µm)

extracted, providing two axes onto which the raw data were projected to give a two-dimensional plot of the taxa and characters. During this process, characters explaining very little of the variance were removed from the original data sets and the analysis was performed without them once again. Only the results of characters X_4, X_5, X_6, X_7, X_8, X_{12}, X_{14}, X_{16}, X_{19} and X_{20} (Tab. 2), explaining most of the variance, are given in this paper.

RESULTS

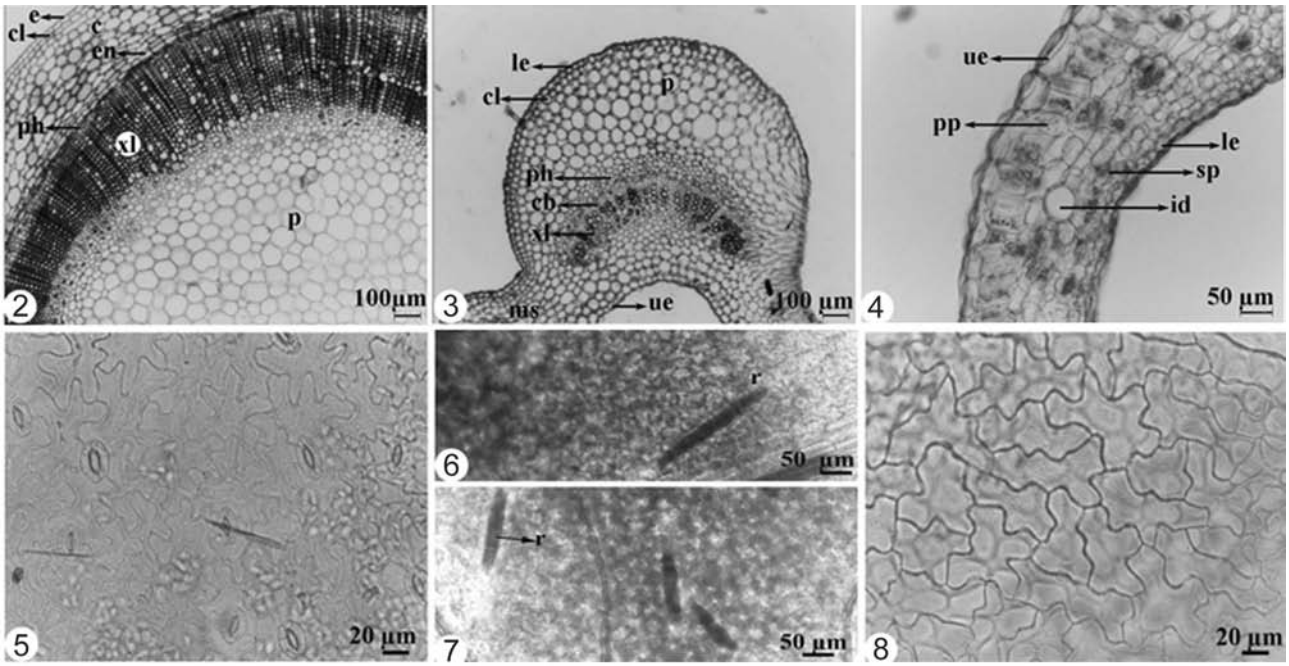
ANATOMICAL CHARACTERS

Epilobium algidum Bieb.: A transverse section of the stem (Fig. 2) showed that the epidermis consists of a single layer of rectangular or orbicular cells; a 1- or 2-layered collenchyma is under the epidermis. The stem cortex (300–350 µm) consists of 8 or 9 layers of usually oval cells and makes up 16% of the stem. The phloem, without sclerenchyma fibers, measures 35–45 µm; xylem 450–500 µm, including solitary or clustered vessels, makes up 29% of the stem radius. The pith includes many intercellular spaces and occupies 50–55% of the stem.

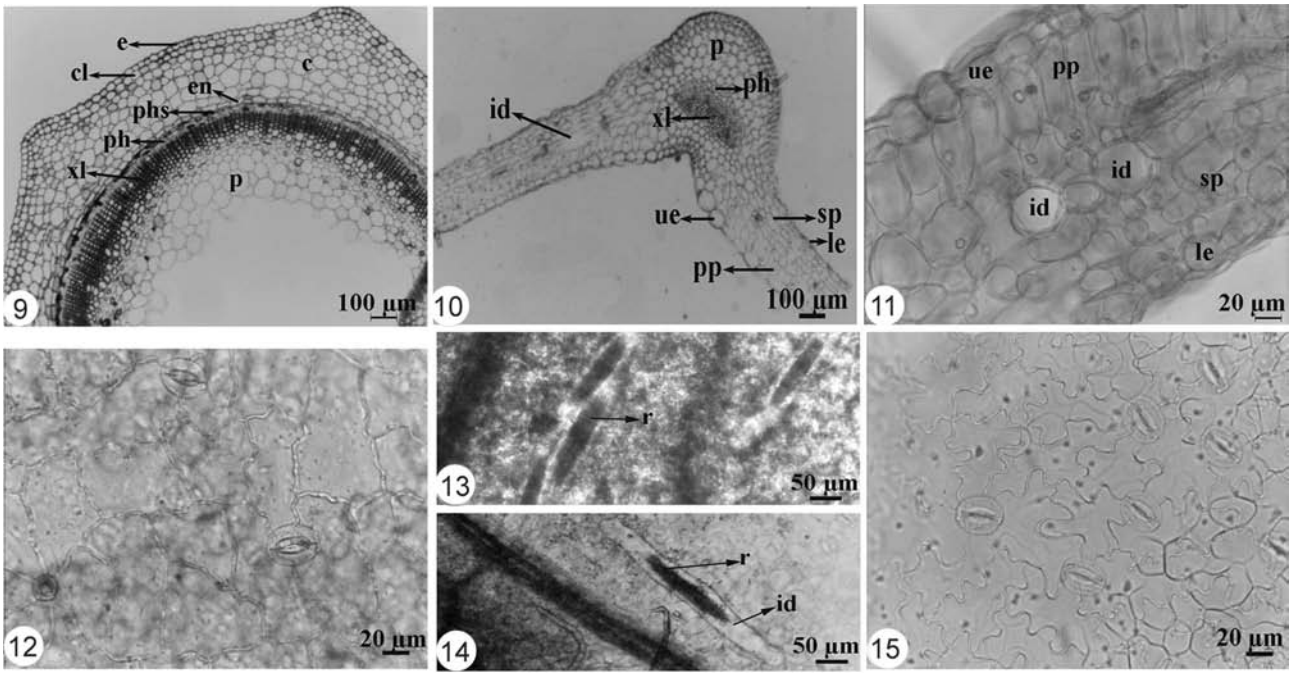
The anatomical features of the midrib, lamina and leaf surfaces were analyzed (Figs. 3–8). The semicircular midrib includes 2 layers of collenchyma close to the epidermal cells and an arc-shaped vascular bundle surrounded by orbicular parenchymatous cells. The cambium is visible (Fig. 3). Idioblasts, including raphids, are sparsely distributed among spongy parenchyma. The mesophyll consists of a monolayer of elongated palisade cells and 4 or 5 layers of isodiametric spongy with large intercellular cavities (Fig. 4). The bifacial leaf is hypostomatic, with anomocytic stomata. The stomata index is 31.7. Upper and lower epidermis cells are similar, with undulate cell walls (Figs. 5–8).

Epilobium palustre L.: A transverse section of the stem (Fig. 9) showed the epidermis to consist of a monolayer of rectangular or orbicular cells. The collenchyma is 2-layered, located close to the epidermis, but 3 or 4 layers of collenchyma can be seen especially at the stem ridges. The cortex (250–260 µm) consists of 4 or 5 layers of usually oval cells with thin walls, and occupies 20–25% of the stem radius. Solitary or clustered sclerenchyma fibers spread in the phloem tissue. Xylem (190–220 µm) occupies 10–15% of the stem radius and bulges slightly at the stem ridges. The pith, with an evident empty central part, occupies 20% of the stem radius.

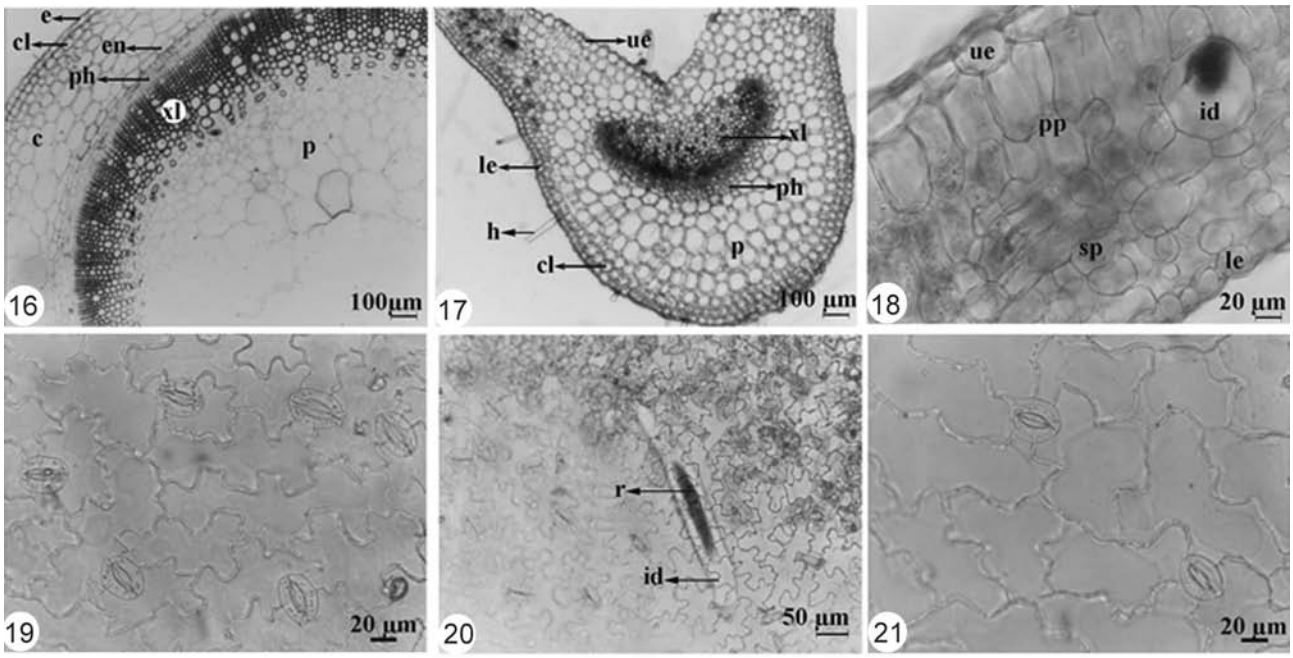
The midrib is semicircular and has a monolayer of collenchyma close to the epidermis. The arc-shaped vascular bundle is surrounded by orbicular and thin-walled parenchymatous cells. Upper epidermal cells are larger than the lower ones (Fig. 10). Idioblasts, including raphids, are distributed among spongy parenchyma cells. The mesophyll consists of a monolayer of elongated palisade cells and 4–5 lay-



Figs. 2–8. *E. algidum*. **Fig. 2**. Transverse section of stem. **Figs. 3,4**. Transverse section of leaf. **Figs. 5–8**. Surface of leaf. e – epidermis; c – cortex; en – endodermis; cl – collenchyma; ph – phloem; xl – xylem; p – pith; le – lower epidermis; ue – upper epidermis; sp – spongy parenchyma; pp – palisade parenchyma; cb – cambium; ms – mesophyll; id – idioblast; r – raphid.



Figs. 9–15. *E. palustre*. **Fig. 9**. Transverse section of stem. **Figs. 10,11**. Transverse section of leaf. **Figs. 12–15**. Surface of leaf. e – epidermis; c – cortex; en – endodermis; cl – collenchyma; ph – phloem; phs – phloem sclerenchyma; xl – xylem; p – pith; le – lower epidermis; ue – upper epidermis; sp – spongy parenchyma; pp – palisade parenchyma; id – idioblast; r – raphid.



Figs. 16–21. *E. ponticum*. Fig. 16. Transverse section of stem. **Figs. 17,18**. Transverse section of leaf. **Figs. 19–21**. Surface of leaf. e – epidermis; c – cortex; en – endodermis; cl – collenchyma; ph – phloem; xl – xylem; p – pith; h – hair; le – lower epidermis; ue – upper epidermis; sp – spongy parenchyma; pp – palisade parenchyma; id – idioblast; r – raphid.

ers of isodiametric spongy parenchymatic cells with large intercellular cavities (Fig. 11). The bifacial leaf has anomocytic stomata on both surfaces. The stomata index is 31.7 for the lower and 27 for the upper surface. Both epidermises have undulate walls (Figs. 12–15).

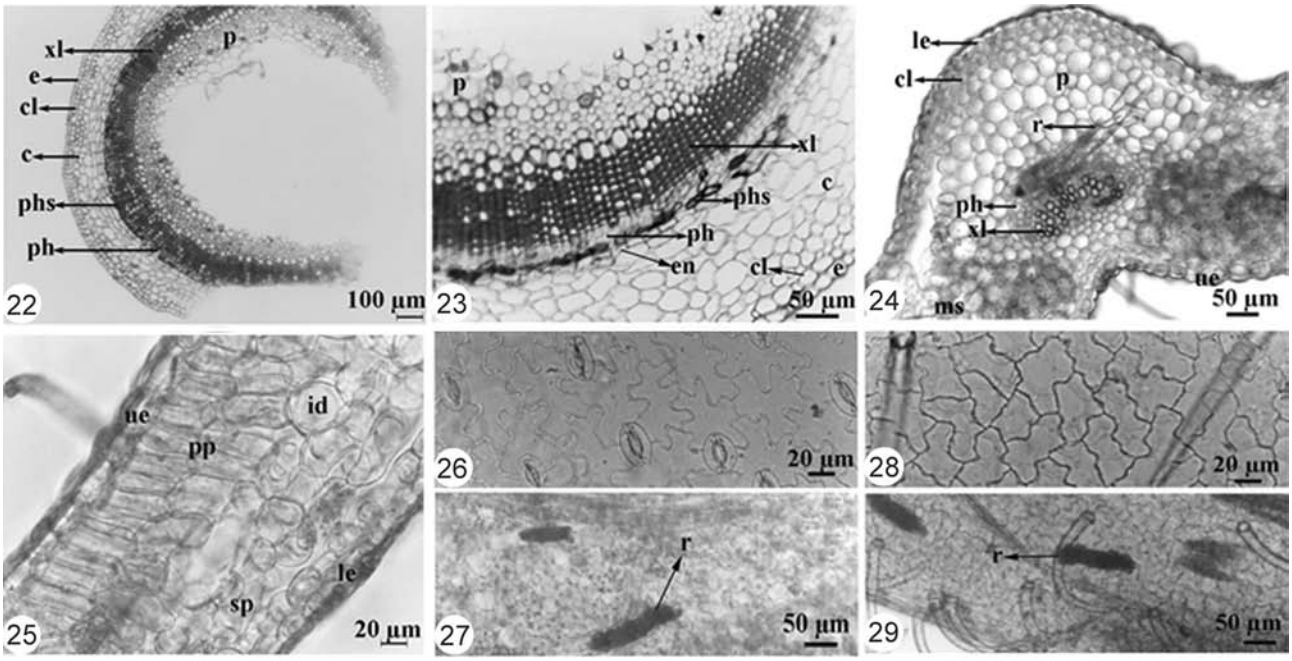
Epilobium ponticum Hausskn.: Transverse sections of the stem (Fig. 16) show that the epidermis consists of a monolayer of thin-walled rectangular cells. The collenchyma beneath the epidermis is 2-layered. The stem cortex (250–300 µm) consists of 4 or 5 layers of usually oval cells and occupies 20% of the stem. Vascular bundles occupy 25% of the stem and are surrounded with an obvious monolayer epidermis. The phloem (40–50 µm) is without sclerenchyma fibers. The xylem (300–330 µm) bulges at the stem ridges. Pith without a free-central part accounts for 40–45% of the radius.

The semicircular midrib has arc-shaped vascular bundles and a distinct 2-layer collenchyma adjacent to the epidermis. The lower epidermis has a few simple hairs, and its cells are slightly smaller than upper epidermal cells (Fig. 17). The mesophyll (255–265 µm) consists of two layers of elongated palisade cells and 4 or 5 layers of isodiametric spongy parenchymatic cells with large intercellular cavities. Very large idioblastic cells are distributed in the palisade and spongy tissue (Fig. 18). The bifacial leaves have anomocytic stomata on both sides; stomata are less dense on the upper than the lower surface. The stoma index is 20.6 for the upper and 16.5 for the lower epidermis (Figs. 19–21).

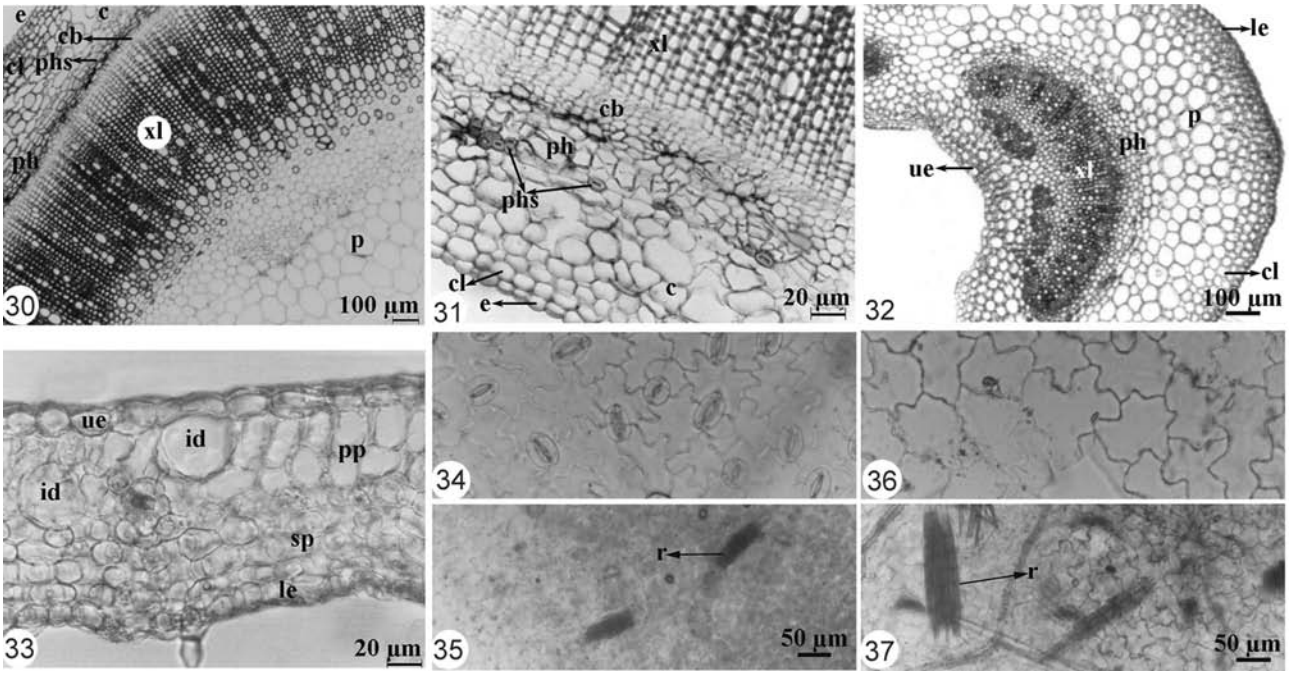
Epilobium confusum Hausskn.: A transverse section of the stem (Fig. 22) showed a uniseriate epidermis consisting of rectangular or orbicular cells, with a monolayer collenchyma beneath it. The stem cortex (140–150 µm) consists of 5 or 6 layers of usually oval cells and occupies 18–20% of the stem radius. The distinct endodermis consists of uniserate rectangular cells. The phloem (20–25 µm) contains solitary or grouped sclerenchymatic fibers (Fig. 23), and the xylem (160–190 µm) occupies 25–30% of the stem radius. The pith, with large cylindrical parenchymatic cells, has an empty central part and occupies 50% of the stem radius.

The midrib is triangular, consisting of 2 rows of collenchyma adjacent to the epidermis. The small semicircular vascular bundle is surrounded by orbicular parenchymatic cells. The mesophyll tissue (180–190 µm) has 2 layers of elongated palisade cells, and 3 or 4 layers of isodiametric spongy cells together with large intercellular spaces and sparse idioblastic cells (Figs. 24, 25). The leaf is bifacial, and both upper and lower epidermal cells have undulate walls (Figs. 26–29), and the upper epidermis is covered with a few simple hairs. The leaf is hypostomatic, with anomocytic stomata. The stomata index is 39.4.

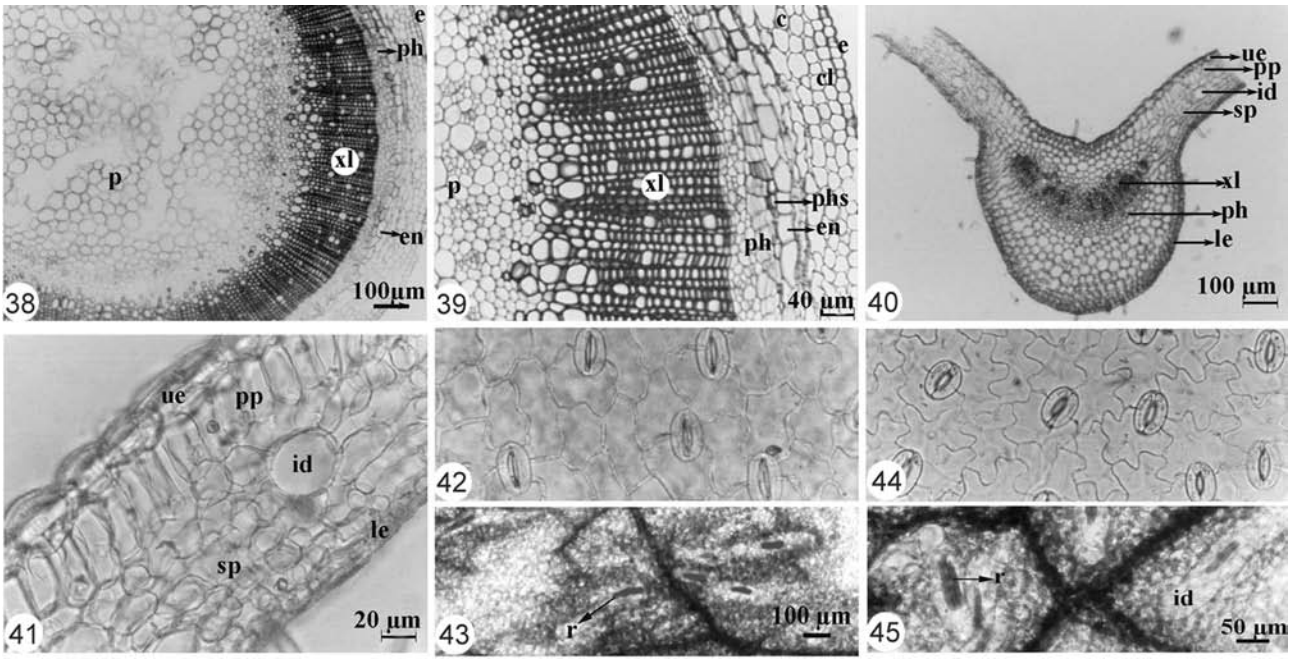
Epilobium hirsutum L.: A transverse section of the middle part of the stem (Fig. 30) shows a uniserate epidermis consisting of large rectangular cells.



Figs. 22–29. *E. confusum*. **Figs. 22,23.** Transverse section of stem. **Figs. 24,25**. Transverse section of leaf. **Figs. 26–29**. Surface of leaf. e – epidermis; c – cortex; en – endodermis; cl – collenchyma; ph – phloem; phs – phloem sclerenchyma; xl – xylem; p – pith; ms – mesophyll; le – lower epidermis; ue – upper epidermis; sp – spongy parenchyma; pp – palisade parenchyma; id – idioblast; r – raphid.



Figs. 30–37. *E. hirsutum*. **Figs. 30,31**. Transverse section of stem. **Figs. 32,33**. Transverse section of leaf. **Figs. 34–37**. Surface of leaf. e – epidermis; c – cortex; cb – cambium; cl – collenchyma; ph – phloem; phs – phloem sclerenchyma; xl – xylem; p – pith; ms – mesophyll; le – lower epidermis; ue – upper epidermis; sp – spongy parenchyma; pp – palisade parenchyma; id – idioblast; r – raphid.



Figs. 38–45. *E. montanum*. **Figs. 38,39.** Transverse section of stem. **Figs. 40,41**. Transverse section of leaf. **Figs. 42–45**. Surface of leaf. e – epidermis; c – cortex; en – endodermis; cl – collenchyma; ph – phloem; phs – phloem sclerenchyma; xl – xylem; p – pith; le- lower epidermis; ue – upper epidermis; sp – spongy parenchyma; pp – palisade parenchyma; id – idioblast; r – raphid.

The cortex is surrounded by a bilayer of collenchyma (100–130 µm) consisting of 4 or 5 rows of usually oval cells, with several intercellular spaces. It occupies 10% of the stem radius. The phloem (50–60 µm) contains solitary or clustered sclerenchymatic fibers (Fig. 31), and the xylem (600–650 µm) occupies 40% of the stem radius. There is a distinct monolayer cambium, and the stem center is filled with large, thin-walled parenchymatous cells.

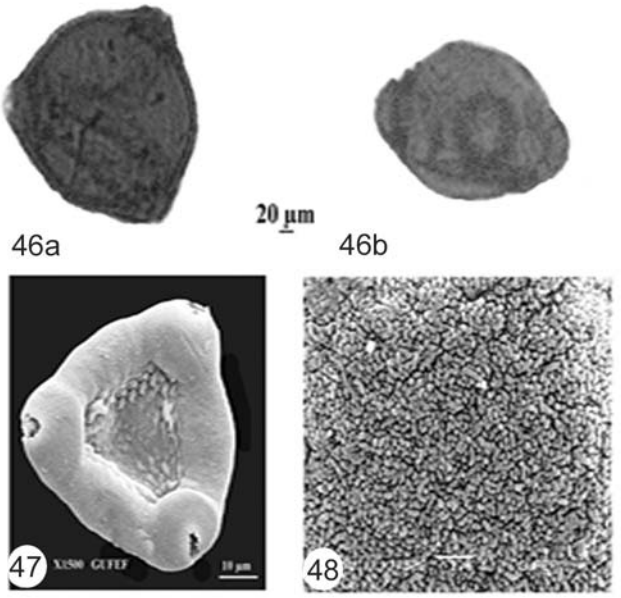
The anatomical features of the midrib and lamina of this species are shown in Figures 32 and 33. The midrib is semicircular and consists of 2 or 3 layers of collenchyma beneath the epidermis. The arc-shaped vascular bundle is surrounded by thin-walled, orbicular parenchymatous cells. The mesophyll (95–100 µm) consists of 2 layers of elongated palisade parenchyma cells, 4 layers of isodiametric spongy cells having large intercellular cavities, and several idioblasts with empty lumens (Fig. 33). The leaf is bifacial, and both upper and lower epidermal cells have undulate walls (Figs. 34–37) and a few simple hairs. The leaf is hypostomatic, with anomocytic or anisocytic stomata. The stomata index is 38.4.

Epilobium montanum L.: The stem in transverse section (Fig. 38) exhibits a monolayer epidermis with orbicular cells and 2 rows of collenchyma close to the epidermis. The cortex (90–100 µm) consists of 3 or 4 rows of usually oval cells and occupies 10–15% of the stem radius. The phloem (40–50 µm) contains a few sclerenchymatic cells (Fig. 39), and the xylem (430–470 µm) occupies 25% of the stem radius. The pith is surrounded by large and circular parenchymatous cells and occupies 50–55% of the stem radius.

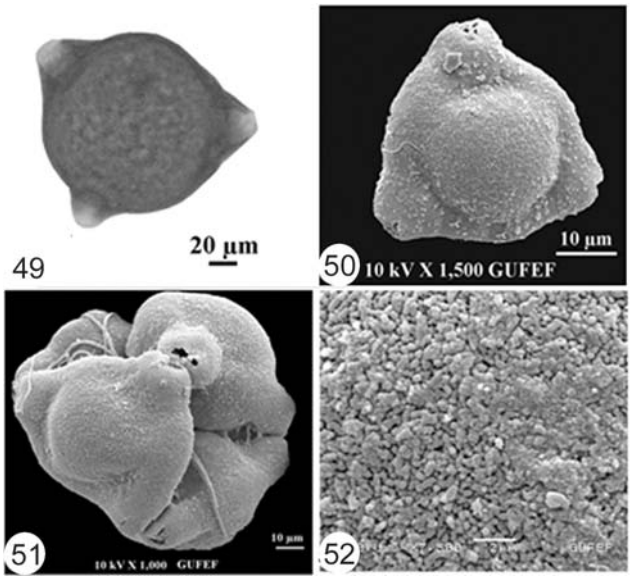
The semicircular midrib consists of 1 or 2 layers of collenchyma adjacent to the epidermis. The arc-shaped vascular bundle is surrounded by thin-walled, orbicular parenchymatous cells (Fig. 40). The mesophyll (115–125 µm) consists of a monolayer palisade, 3 or 4 layers of isodiametric spongy parenchyma with large intercellular cavities, and several idioblasts (Fig. 41) including raphid crystals (Figs. 43, 45). The upper epidermal cells are bigger than the lower ones (Fig. 41). The leaf is bifacial, and both upper and lower epidermal cells have undulate walls (Figs. 42, 45) and have anomocytic or anisocytic stomata. The stoma index is 15.4 for the lower and 24.1 for the upper surface.

POLLEN CHARACTERS

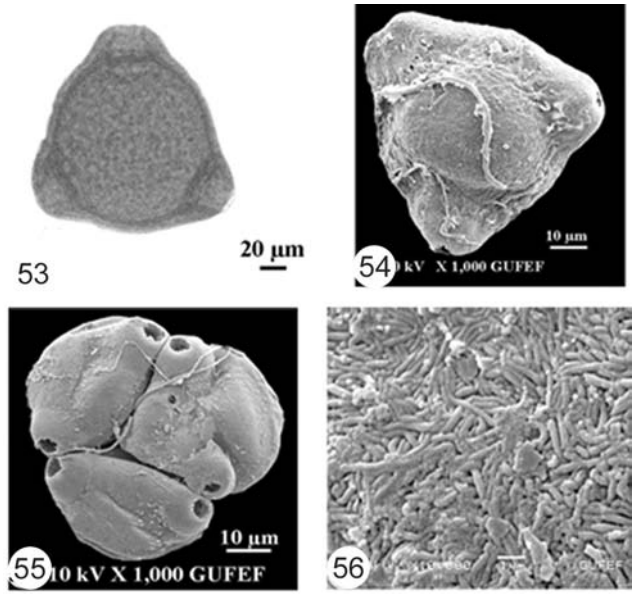
Epilobium algidum Bieb.: The pollen grains are radially symmetrical, isopolar, and generally shed in tetrads, rarely monads. Pollen shape is suboblate, with the polar axis 66.4 µm and equatorial axis 96.2 µm. The outline is circular in equatorial optical section and triangular in meridional optical section (Fig. 46a,b). The grains are 3-zonoporate.



Figs. 46–48. Pollen grains of *E. algidum*. **Fig. 46.** (**a**) Polar view, (**b**) Equatorial view. **Fig. 47**. Polar view. **Fig. 48.** Exine ornamentation.



Figs. 49–52. Pollen grains of *E. palustre*. **Figs. 49, 50.** Polar view. **Fig. 51.** Microspore tetrad. **Fig. 52.** Exine ornamentation.



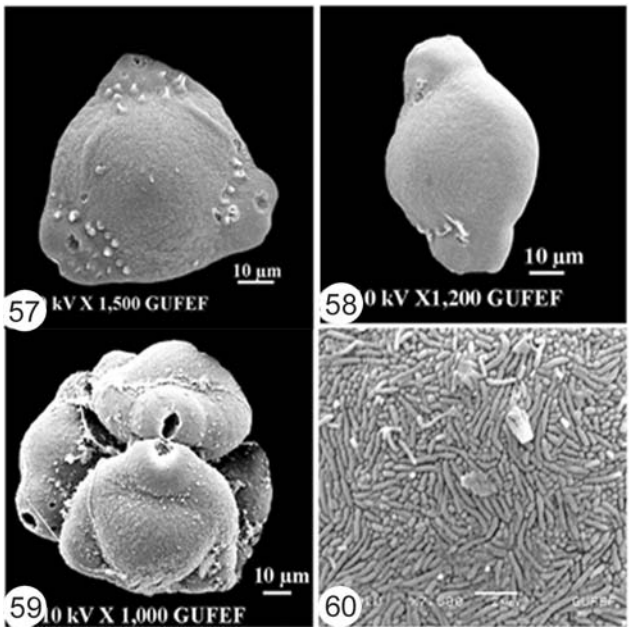
Figs. 53–56. Pollen grains of *E. ponticum*. **Figs. 53, 54**. Polar view. **Fig. 55.** Microspore tetrad. **Fig. 56**. Exine ornamentation.

The apertural membrane is generally psilate and rarely granulate (Fig. 47). The exine is tectate and 4.6 µm thick. The ectexine (2.8 µm) is thicker than the endexine (1.8 µm). The intine is 1.9 µm thick on the equatorial axis. The exine shows tumescence at the aperture, and ornamentation is baculate (Fig. 48).

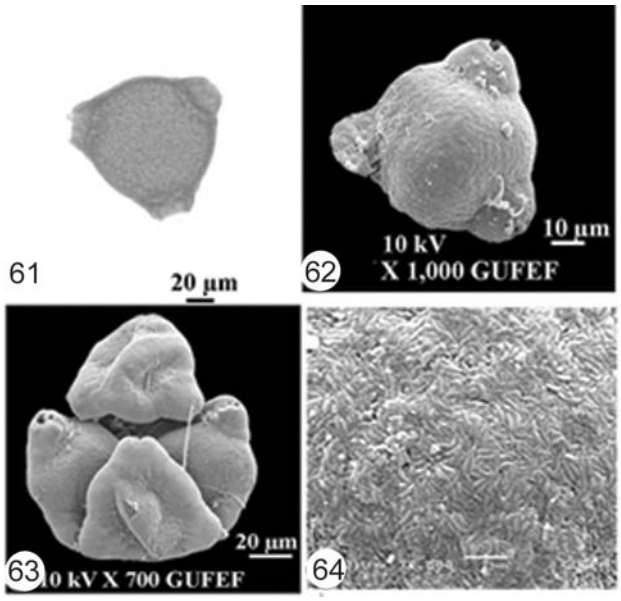
Epilobium palustre L.: The pollen grains are radially symmetrical, isopolar, and generally shed in monads, rarely tetrads. Pollen shape is suboblate, with the polar axis 69.2 µm and equatorial axis 87.6 µm. The outline is triangular in meridional optical section (Fig. 49). The grains are 3-zonoporate. The apertural membrane is generally psilate and rarely granulate (Figs. 50, 51). The exine is tectate and 4.1 µm thick. The ectexine (2.4 µm) is thicker than the endexine (1.7 µm). The intine is 1.3 µm thick on the equatorial axis. The exine shows tumescence at the aperture, and ornamentation is baculate (Fig. 52).

Epilobium ponticum Hausskn.: The pollen grains are radially symmetrical, isopolar, and shed in monads, rarely tetrads. Pollen shape is suboblate, with the polar axis 71.7 µm and equatorial axis 94 µm. The outline is triangular in meridional optical section (Fig. 53). The grains are 3-zonoporate. The apertural membrane is generally psilate and rarely granulate (Figs. 54, 55). The exine is tectate and 5.3 µm thick. The ectexine (3.6 µm) is thicker than the endexine (1.7 µm). Intine thickness is 1.4 µm on the equatorial axis. The exine shows tumescence at the aperture, and ornamentation is striate (Fig. 56).

Epilobium confusum Hausskn.: The pollen grains are radially symmetrical, isopolar, and shed in monads, rarely tetrads. Pollen shape is suboblate, with the polar axis 67.9 µm and equatorial axis 94.5 µm. The outline is circular in equatorial optical section and triangular in meridional optical section (Figs. 57, 58). The grains are 3-zonoporate. The apertural membrane is generally psilate and rarely



Figs. 57–60. Pollen grains of *E. confusum*. **Fig. 57**. Polar view. **Fig. 58**. Equatorial view. **Fig. 59**. Microspore tetrad. **Fig. 60**. Exine ornamentation.

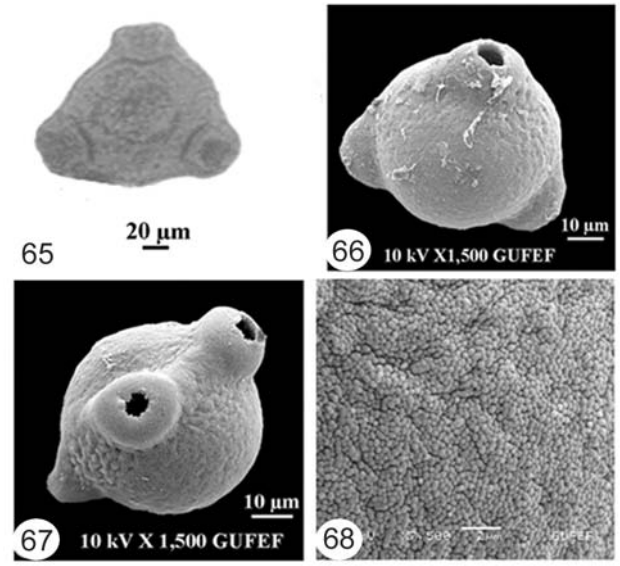


Figs. 61–64. Pollen grains of *E. hirsutum*. **Figs. 61, 62.** Polar view. **Fig. 63**. Microspore tetrad. **Fig. 64**. Exine ornamentation.

granulate (Figs. 58, 59). The exine is tectate and 4.4 µm thick. The ectexine (2.7 µm) is thicker than the endexine (1.7 µm). Intine thickness is 1.9 µm on the equatorial axis. The exine shows tumescence at the aperture, and ornamentation is striate (Fig. 60).

Epilobium hirsutum L.: The pollen grains are radially symmetrical, isopolar, and usually shed in monads, rarely in tetrads. Pollen shape is suboblate, with the polar axis 69.8 µm and equatorial axis 94 µm. The outline is circular in equatorial optical section and obtuse-triangular in meridional optical section (Fig. 61). The grains are 3-zonoporate. The apertural membrane is generally psilate and rarely granulate (Figs. 62, 63). The exine is tectate and 5 µm thick. Th ectexine (3.3 µm) is thicker than the endexine (1.7 µm). Intine thickness is 1.7 µm on the equatorial axis. The exine shows tumescence at the aperture, and ornamentation is baculate (Fig. 64).

Epilobium montanum L.: The pollen grains are radially symmetrical, isopolar, and usually shed in monads, rarely in tetrads. Pollen shape is spheroid, with the polar axis 78.1 µm and equatorial axis 91.2 µm. The outline is circular in equatorial optical section and and obtuse-triangular in meridional optical section (Fig. 65). The grains are 3-zonoporate. The apertural membrane is generally psilate and rarely granulate (Figs. 66, 67). The exine is tectate and 4.4 µm thick. The ectexine (2.9 µm) is thicker than the endexine (1.5 µm). Intine thickness is 1.5 µm on the equatorial axis. The exine shows tumescence at the aperture, and ornamentation is baculate (Fig. 68).



Figs. 65–68. Pollen grains of *E. montanum*. **Figs. 65, 66**. Polar view. **Fig. 67**. Equatorial view. **Fig. 68**. Exine ornamentation.

NUMERICAL ANALYSIS

The dendrogram resulting from UPGMA based on 43 variables (27 related to anatomy and 16 related to palynology) is represented in Figure 69. Six *Epilobium* species fall into two major clusters, one with two of the six species, and the other with the remaining ones. The first group includes *E. algidum* and *E. plasture*, linked to each other at 0.8% dis-

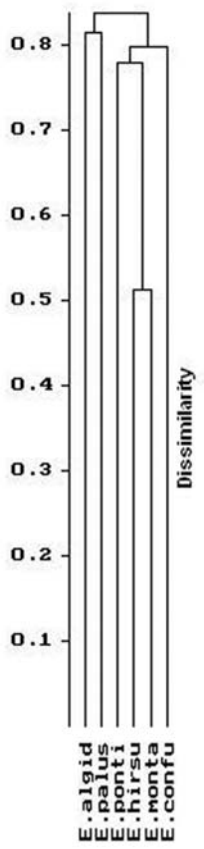


Fig. 69. Cluster analysis – UPGMA.

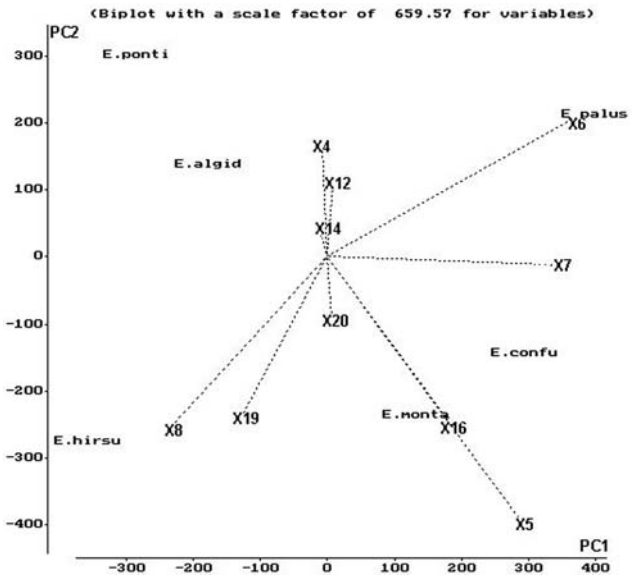


Fig. 70. Principal component analysis of 6 taxa and 10 variables projected onto PC1 and PC2. For explanations of variable numbers see Table 2.

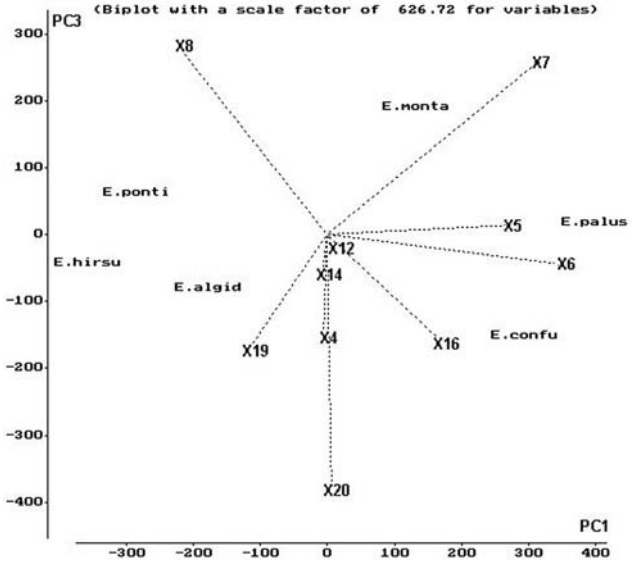


Fig. 71. Principal component analysis of 6 taxa and 10 variables projected onto the PC1 and PC3. For explanations of variable numbers see Table 2.

similarity level. The second larger cluster divides into smaller clusters, with *E. hirsutum* and *E. montanum* are nested in a tight cluster, linked at 0.5% dissimilarity level. To determine which traits are important in explaining total variation among the examined species, PCA analysis was performed on the raw data given in Table 2. Most of the traits are not important in explaining total variation, so only the PCA results for ten characters are given in Figures 70 and 71. The taxa and the variables of the first two components are also given. Table 3 gives eigenvalues as percentages of the explained variance based on both the selected 10 traits and also 43 eigenvalues as percentages of the variance explained by the selected 10 variables and also by all 43. Only the first three components were taken into account because of their high eigenvalues. The first, second and third components accounted for 53.74%, 32.72% and 7.60% of the variance.

DISCUSSION

This study sought to provide useful anatomical and palynological information and additional perspective to the systematics of the examined *Epilobium* taxa. This is the first anatomical and palynological study of Turkish representatives of *Epilobium*.

Stem anatomy is similar in the examined taxa, but xylem width and the presence and distribution of sclerenchymatic cells in the phloem vary between the taxa. The xylem forms a continuous cylinder in all the examined taxa, as indicated by Metcalfe and Chalk (1950); they stated that the xylem is traversed

by narrow rays in most *Epilobium* taxa, but this was not observed in our study. Xylem width in *E. hirsutum* (600–650 μm) obviously differs from the other examined species (Fig. 30).

The distribution and presence of sclerenchymatic tissue in the stem cortex and phloem has considerable taxonomic value (Canne-Hilliker and Kampny, 1990). It occurs as solitary or grouped fibers in some taxa. While the fibers spread as a layer between cortex and phloem in *E. palustre* (Fig. 9), they are solitary or 2–5 (7) grouped in *E. confusum, E. hirsutum* and *E. montanum* (Figs. 23, 31, 39). In contrast, *E. algidum* and *E. ponticum* do not possess fibers (Figs. 2, 16). Metcalfe and Chalk (1950) and Makbul et al. (2006) reported the fibers as taxonomic characters in Onagraceae and *Scrophularia*, respectively.

Leaves anatomy varies greatly and provides many systematically significant characters (Carlquist, 1961). We found that the presence and distribution of idioblasts are particularly significant characters in the examined taxa. While all examined taxa posses idioblasts in their mesophyll, their size and number vary significantly. The average number of idioblasts per mm is 25 in *E. confusum*, 6 in *E. ponticum*, and between 9 and 15 in the others. Although idioblasts supply additional taxonomic information for grouping, as indicated by Makbul et al. (2006), this character was not seen as an important feature in the numerical analysis. Among the other foliar peculiarities observed in this study, the number of rows and the width of palisade parenchyma, and the presence of hairs on leaves were found to be important in the examined taxa. These findings accord with the information given by Metcalfe and Chalk (1950) for Onagraceae.

All the leaves are bifacial and have anomocytic stomata. The stomata index is 15.4 in *E. montanum*, 39.4 in *E. confusum*, 38.4 in *E. hirsutum*, and ranges from 20 to 32 in the other examined taxa. While the stomata index varies from species to species, it is also well known that it is an environmentally influenced anatomical character, so it is not so useful in grouping the examined taxa. The leaves of *E. algidum, E. confusum* and *E. hirsutum* are hypostomatic. The other species are amphistomatic. Thus the presence of stomata on the adaxial or abaxial surface is an important diagnostic character, but above the species level.

The palynological data presented here reinforce the close relationship among *Epilobium* species. Pollen grains of *E. algidum* are generally shed in tetrads, but the other examined species commonly have monad pollen grains. Our results are in accordance with Punt et al. (2003), who reported that pollen grains of *Epilobium* are usually shed in monads, rarely in tetrads, and that the monads are 3-zonoporate, subisopolar and radially symmetric.

TABLE 3. Percentage of variance and square roots of eigenvalues accounted for by first three components

Components	Based on 43 variables		Based on 10 variables	
	Variance (%)	Square roots of eigenvalues	Variance (%)	Square roots of eigenvalues
PC1	53.32	318.45	53.74	317.95
PC2	7.71	248.17	32.72	248.11
PC3	4.44	120.83	7.60	119.55
Total	65.47	–	94.06	–

Mitroiu (1963) and Brown (1967) noted that the pollen is shed in tetrads and that this character is very common in the genus *Epilobium*. In the present study, tetrads and monads were present together. The polar/equatorial (P/E) ratio is 0.85 and pollen shape is spheroid in *E. montanum*; in the others the ratio ranges from 0.69 to 0.78 and pollen shape is suboblate. In some *Epilobium* species, Punt et al. (2003) observed oblate to suboblate pollen, and ornamentation psilate in LM and rugulate in SEM. In our study we found striate ornamentation in *E. confusum* and *E. ponticum*, and baculate ornamentation in others. We conclude that pollen shape and ornamentation vary among the examined taxa and have taxonomic value in delimiting them.

The anatomical and palynological properties of the examined *Epilobium* taxa were analyzed by numerical methods in order to determine the relevance of several traits in *Epilobium* systematics. In clustering analysis, the cophenetic correlation coefficient (r_{cs}) was calculated in order to assess how well the dendrogram represented the underlying matrix of resemblances. The simplicity of r_{cs} has led to its extensive application (Sneath and Sokal, 1973). It has generally been found to vary from 0.6 to 0.95, depending on the methods used to produce the dendrogram and the nature of the differences among the specimens classified. Our dendrogram had a cophenetic correlation of 0.84, suggesting that the dendrogram provides an accurate representation of the resemblances. A cross line of the dendrogram resulting from UPGMA at 0.5 dissimilarity divided the dendrogram in six groups corresponding to the four taxa of *Epilobium* conventionally identified. Hence, we suggest that this clustering method is suitable for classifying the genus at species level. As seen in Figure 69, *E. hirsutum* and *E. montanum* are linked at a similarity level higher than that of the last four examined taxa. *E. algidum* and *E. palustre* are clustered in a separate minor group, but with high dissimilarity.

There are six taxa groups corresponding to the four examined taxa, separated from each other on the PC1 vs PC2 and PC1 vs PC3 axes (Figs. 70, 71). The three components together account for 90.96%

of variation when the 10 most important variables are used, and 65.47% when all 43 variables are (Tab. 3). While the first component accounts for 52.32% of the variation based on 43 variables, it explains 53.74% of it based on the 10 selected ones. The second component based on 43 variables accounts for 7.21%, so that together they account for 61.03%, but they explain 90.96% based on the 10 variables. This shows that the first three components explain most of the total variation among the examined *Epilobium* species based on the 10 characters selected. That is why only the results based on the 10 variables are given here. PCA analysis also shows that some of the 43 examined traits are more important in delimiting the examined *Epilobium* species: width of cortex, pith, xylem and palisade parenchyma (μm), and average number of cortex and pith cells, trachea, lower and upper epidermal cells. This suggests that anatomical features are more important than palynological ones in explaining variation among the examined *Epilobium* taxa.

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