

Available online at www.dergipark.gov.tr/beuscitech

Journal of Science and Technology

E-ISSN 2146-7706



Using of light and scanning electron microscopy for pollen morphology of some *Epilobium* (Onagraceae) taxa and Its taxonomic importance

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ARTICLE INFO

Article history:

Received 29 September 2021

Received in revised form 21 October 2021

Accepted 04 November 2021

Keywords:

Epilobium,
LM,
Onagraceae,
Pollen morphology,
SEM

ABSTRACT

In this study, pollen morphologies of eleven species of the *Epilobium* L. genus belonging to Onagraceae family were investigated under light and scanning electron microscopy. Solutions to taxonomic problems of these species were sought with palynological information. The characteristics of the genus *Epilobium* L. studied as a result of palynological studies are as follows: It has been determined that their pollen grains have tetrad, angulaperturate, 3-zonoporate (rarely 2,4 porate), suboblate and oblate-spheroidal pollen shapes, and have baculate, baculate-rugulate, and rugulate ornamentation.

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1. Introduction

The Onagraceae family having very rich and widespread genera and species are represented by nearly 20 genera and 656 species in the world (Raven, 1962a; Heywood et al., 2007). The genus *Epilobium* L. (Onagraceae) contains about 200 species that grow in the subarctic, temperate, and subantarctic regions in the world (Walter et al. 2007). *Epilobium* are mostly herbs. However, few of them are annual or perennial subshrubs. In total, 22 *Epilobium* species have been reported in Turkey (Okur 2019). *Epilobium* is a very difficult genus due to its very similar appearance and high probability of hybridization among almost all taxa (Makbul 2008).

Palinomorphology of Onagraceae has been examined by many authors (Erdtman, 1952; Mitroiu, 1963; Ting, 1966; Brown, 1967; Skvarla, et al., 1978-2008; Keri & Zetter, 1992; Praglowksi et al., 1994; Rowley & Claugher, 1996; and Rowley & Skvarla, 2006). Mosquin (1966) examined two subspecies of *E. angustifolium* according to their morphology; *E. angustifolium*

L. subsp. *circumvagum* Mosquin taxon is tetraploid (n=36) and *E. angustifolium* L. subsp. *angustifolium* is diploid (n=18) and hexaploid (n=54). Tetraploid taxa with three and four pores, and diploid taxa having pollens with three pores are found together. Brown, (1967) stated that in the Epilobieae tribe, which also includes the *Epilobium* pollen grains are seen as either monads or tetrahedral tetrads. The pollen of tetraploid taxa is larger than the pollen of other taxa whereas, pollen grains with four pores are denser in hexaploid taxa. Punt et al., (2003) comprehensively studied pollen morphology of the Onagraceae from Northwest Europe. Skvarla et al., (2008) examined pollen morphology of *Epilobium luteum* of Onagraceae belonging to tribe Onagreae. Makbul et al., (2008) studied the anatomical and pollen morphological characters of the genus *Epilobium* from Northwest Anatolia and reported that anatomical features are more important than palynological features for explaining the variation among these taxa. Skvarla et al., (2008) observed that the pollen grains of the genus *Epilobium* has both monad and tetrahedral tetrad structure.

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Punt et al., (2003) and Makbul et al., (2008) determined that the pollen shape of the *Epilobium* taxon was suboblate or oblate and the pollen shape contributed significantly to the differentiation of the *Epilobium* taxon. Erdtman, 1960; Punt et al., 2003 revealed that there is a fine connection between the tetrad and monad pollen spreads by breaking this connection with the acetolysis method. Rahimi et al., (2018) determined that radial symmetrical, tetrahedral tetrad, trizonoporate, rugulate, granulate, verrucate, and striate ornamentations were observed in pollens and that these family members did not differ significantly in terms of characteristics such as pollen size, shape, and pore structure. Perveen and Qaiser (2013) determined that the pollen distribution of *Epilobium* is generally tetrahedral tetrad and rarely monad.

The aim of the study is to perform pollen morphology analyses of 11 different taxa belonging to *Epilobium* by using light and scanning electron microscopy methods and to clarify the usefulness of these features in terms of taxonomic implications.

2. Materials and Methods

Plant specimens was obtained from the specimens of Gazi University herbarium (GAZI) and Bitlis Eren University Herbarium. The list of voucher specimens is deposited in GAZI and Bitlis Eren University Herberia.

Specimens investigated.

***E. hirsutum* L.:** B8-Bitlis: Bitlis Ağačköprü Village and stream sides, 26.07.2013, 38° 20' 11.95" K, 42° 00' 10.88" D, 1350-1450m, M. Karataş 1304, Det.: M. Kurşat. ***E. parviflorum* Schreb.:** B8-Bitlis: Bitlis, Kambos Mountain north slope, 18.07.2014, 38° 19' 23.26" K, 41° 59' 42.29" D, 1800-1950m, M. Karataş 2318, Det.: M. Kurşat. ***E. minutiflorum* Hausskn.:** B8-Bitlis: Bitlis, East of Mount Kambos, 18.06.2014, 38° 19' 29.58" K, 42° 00' 33.33" D, 1400-1600 m, M. Karataş 2131, Det.: M. Kurşat. ***E. montanum* L.:** A4-Çankırı: Çankırı- Artacalar, Dumanlı Mountain, 1350 m, 05.07.1991 A. Duran 1236 (GAZI) ***E. confusum* Hausskn.:** B8-Bitlis: Bitlis, East of Mount Kambos, 18.06.2014, 38° 19' 29.58" K, 42° 00' 33.33" D, 1400-1600 m, M. Karataş 2088, Det.: M. Kurşat. ***E. ponticum* Hausskn.:** B8-Bitlis: Bitlis, Mount Kambos south, 15.03.2014, 38° 17' 52.83" K, 41° 59' 15.31" D, 1240- 1650m, M. Karataş 1553, Det.: M. Kurşat. ***E. gemmascens* C.A.Mey.:** B8-Bitlis: Bitlis, Kambos Mountain north slope 08.07.2013, 38° 19' 23.26" K, 41° 59' 42.29" D, 1800-1950m, M. Karataş 1188, Det.: M. Kurşat. ***E. anagallidifolium* Lam.:** B8-Bitlis: Bitlis, Kambos Mountain north slope 03.07.2013, 38° 19' 23.26" K, 41° 59' 42.29" D, 1800-1950m, M. Karataş 1060, Det.: M. Kurşat. ***E. palustre* L.:** A8 Rize: Rize İkizdere, Başköy, 24.07.1984 A. Güner 6052 (GAZI). ***E. anatolicum* Hausskn. subsp. anatolicum.:** C6 Maraş: Maraş-Berit mountain, Karagöl, Yataz slope 2600 m, 25.07.1992 Z. Aytac, 5531 (GAZI). ***E. anatolicum* subsp. prionophyllum (Hausskn.) P.H.Raven:** A8 Rize: Rize İkizdere, Başköy, 18.06.1986, A.Güner 6969 (GAZI).

For light microscopy (LM) analysis, The pollen grains were prepared for light (LM) and scanning microscopy (SEM) by the standard methods described by Wodehouse (1935). For light microscopy, the pollen grains were mounted in Safranin glycerin jelly and observations were made with a Olympus BX-31 microscope under (E40, 0.65) and oil immersion (E100, 1.25), using 10x eye piece. Measurements were performed on at least 30 pollen grains per specimen for each morphological character (Polar axis (P), equatorial diameter (E), Plg (Porus length), Plt (porus width), mesopodium, apopodium, annulus length, annulus diameter, costae, exine and intine with LM using an oil immersion 100U objective lens.

For SEM studies, pollen grains were dried, mounted on stubs, coated with gold by sputter coater, and the SEM examination was carried using a ZEISS Supra 55 Scanning Electron Microscope at the SEM Laboratory of the Central Research Laboratory (MERLAB), Yuzuncu Yil University, Van. In general, the terminology used is in accordance with Punt et al. (2007) and Hesse et al. (2009).

3. Results

The palynological properties of the examined *Epilobium* taxa are summarized in Table 1. Representative pollen grains are illustrated in Figures 1–3.

3.1. Size, symmetry, and shape

The pollen grains of the investigated species are dispersed as tetrad. They can be described as bilateral symmetrical and subisopolar based on SEM and LM observations. The shape of the pollen grains in equatorial view are suboblate, and oblate-spheroidal, whilst their shape in polar view is triangular-obtus. The pollen grains of the genus are oblate-spheroidal, suboblate (*E. anatolicum* subsp. *anatolicum*, *E. palustre*, and *E. confusum*). The polar axis varies from 57.52-87.33 µm and the equatorial diameters from 65.42-95.23 µm. Amb diameter 62.50-92.74 µm. Mesopodium (M) 36.78-62.08 µm. Apopodium (Ap) 37.15-60.07 µm. Their dimensions are smaller in *E. palustre* and larger in *E. hirsutum* (Table 1; Figures 1-3).

3.2. Apertures

In the investigated taxa, there are eleven taxa with trizonoporate (rarely 2,4 porate), apertures at corners (angulaaperturate) (Table 1). The porus is long (10.81-22.03 µm) and broad (11.97-22.66 µm); The shape of porus is circular. The apertural membrane is generally psilate and rarely granulate. Annulus thickness (An-th) 4.25-6.35 µm. Annulus height (An-h) 14.29-23.58 µm (Table 1; Figures 1-3).

3.3. Exine, intine and Ornamentations

The exine ranges from 1.78 to 2.68 µm. Ectexine is thicker than endexine without costae and the intine is 0.39-0.69 µm thick. The exine shows tumescence at the aperture. Costae thickness

(C) 2.45-3.35 μm . Exine sculpturing, showed three distinct types of surface structures: Baculate (the common type), baculate-rugulate (*E. anatolicum* subsp. *prionophyllum*), and rugulate (*E. montanum*) (Table 1; Figures 1-3).

Table 1. Summary of pollen morphological data for the *Epilobium* species examined.

Taxa	P	E	P/E	Plg	Plt	Ex	In
<i>E. hirsutum</i>	87.33±11.09	95.23±10.54	Oblate-spheroidal	22.03±2.99	22.66±2.67	2.68±0.36	0.66±0.18
<i>E. parviflorum</i>	72.03±5.95	79.83±6.43	Oblate-spheroidal	18.10±2.96	17.57±2.85	2.47±0.40	0.45±0.17
<i>E. montanum</i>	82.52±3.09	94.30±3.13	Oblate-spheroidal	19.42±1.76	19.58±1.95	1.78±0.18	0.56±0.11
<i>E. anatolicum</i> subsp. <i>anatolicum</i>	59.87±3.41	67.45±2.46	Suboblate	16.01±1.55	15.97±1.20	2.29±0.57	0.63±0.17
<i>E. anatolicum</i> subsp. <i>prionophyllum</i>	73.73±4.23	80.80±3.76	Oblate-spheroidal	20.51±1.94	22.23±2.64	1.84±0.32	0.50±0.14
<i>E. palustre</i>	57.52±3.48	65.42±4.83	Suboblate	10.81±1.33	11.97±1.25	2.14±0.43	0.57±0.12
<i>E. minutiflorum</i>	63.17±7.85	68.00±7.98	Oblate-spheroidal	14.01±3.07	14.40±2.50	2.34±0.25	0.39±0.13
<i>E. confusum</i>	68.17±4.50	76.67±4.31	Suboblate	16.30±1.99	17.40±2.03	2.10±0.22	0.41±0.18
<i>E. ponticum</i>	71.23±4.69	76.03±4.84	Oblate-spheroidal	19.66±1.72	20.33±1.49	2.35±0.19	0.69±0.16
<i>E. gemmascens</i>	79.86±7.84	87.96±7.50	Oblate-spheroidal	14.87±2.36	16.77±2.35	2.21±0.24	0.51±0.15
<i>E. anagallidifolium</i>	67.20±2.34	74.50±2.69	Oblate-spheroidal	14.60±1.10	14.43±1.25	2.30±0.33	0.54±0.09

Taxa	An-th	An. h.	Amb	M	Ap	C	Orn.
<i>E. hirsutum</i>	4.61±0.90	21.49±2.01	90.09±6.54	61.86±3.73	59.40±1.42	2.49±0.48	Baculate
<i>E. parviflorum</i>	4.98±0.71	18.93±1.34	83.92±5.83	46.41±2.38	48.10±0.92	3.25±0.26	Baculate
<i>E. montanum</i>	6.13±0.52	20.39±2.01	92.74±4.12	62.08±3.24	60.07±1.27	3.35±0.46	Rugulate
<i>E. anatolicum</i> subsp. <i>anatolicum</i>	5.54±0.72	16.56±1.26	65.75±2.75	36.78±2.09	37.15±0.90	3.11±0.52	Baculate
<i>E. anatolicum</i> subsp. <i>prionophyllum</i>	6.35±1.01	18.29±1.73	84.50±5.57	46.52±1.98	48.42±2.43	2.87±0.45	Baculate-rugulate
<i>E. palustre</i>	5.84±0.70	14.29±1.20	62.50±5.30	37.14±0.78	39.02±0.94	2.65±0.46	Baculate
<i>E. minutiflorum</i>	5.27±1.39	15.48±2.48	70.36±5.30	43.07±1.18	44.82±2.34	2.79±0.58	Baculate
<i>E. confusum</i>	4.98±0.82	17.69±1.13	78.19±3.77	40.24±0.62	41.43±3.08	2.55±0.20	Baculate
<i>E. ponticum</i>	4.25±1.04	20.28±0.98	77.42±4.17	50.72±1.08	48.43±2.66	2.45±0.31	Baculate
<i>E. gemmascens</i>	6.01±0.74	23.58±1.01	89.24±3.29	55.08±2.03	56.73±1.99	2.72±0.54	Baculate-rugulate
<i>E. anagallidifolium</i>	4.50±0.62	20.25±1.40	72.02±2.94	50.72±1.08	53.01±1.08	2.75±0.63	Baculate

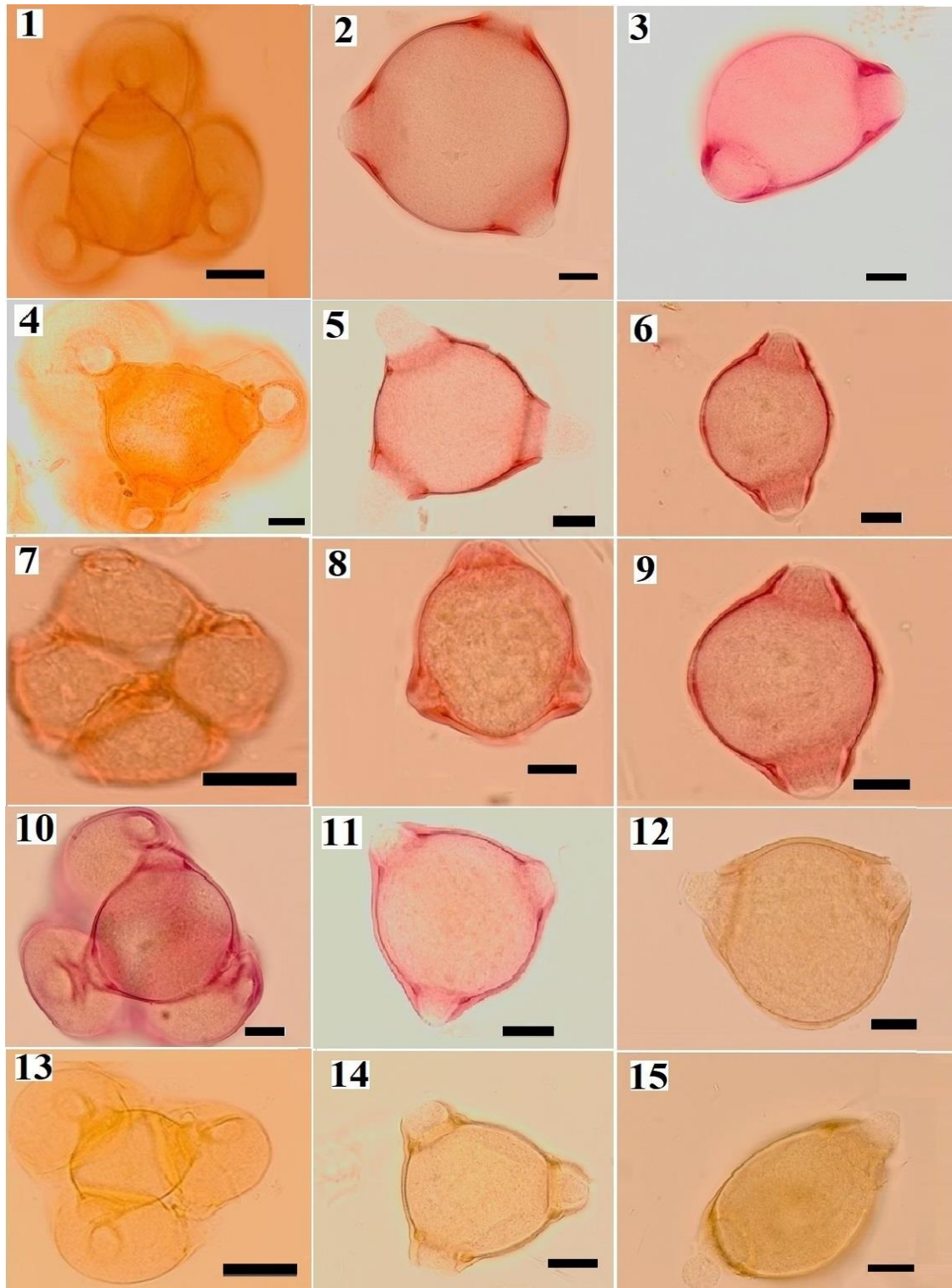


Figure 1. Pollen grains LM photos of studied taxa: tetrad, polar and equatorial view respectively; **(1-3)** *Epilobium hirsutum*, **(4-6)** *E. parviflorum*, **(7-9)** *E. montanum*, **(10-12)** *E. anaticum* subsp. *anaticum*, **(13-15)** *E. anaticum* subsp. *prionophyllum* (Scale bar 20 μm) and **(1,7,13)** Scale bar 50 μm).

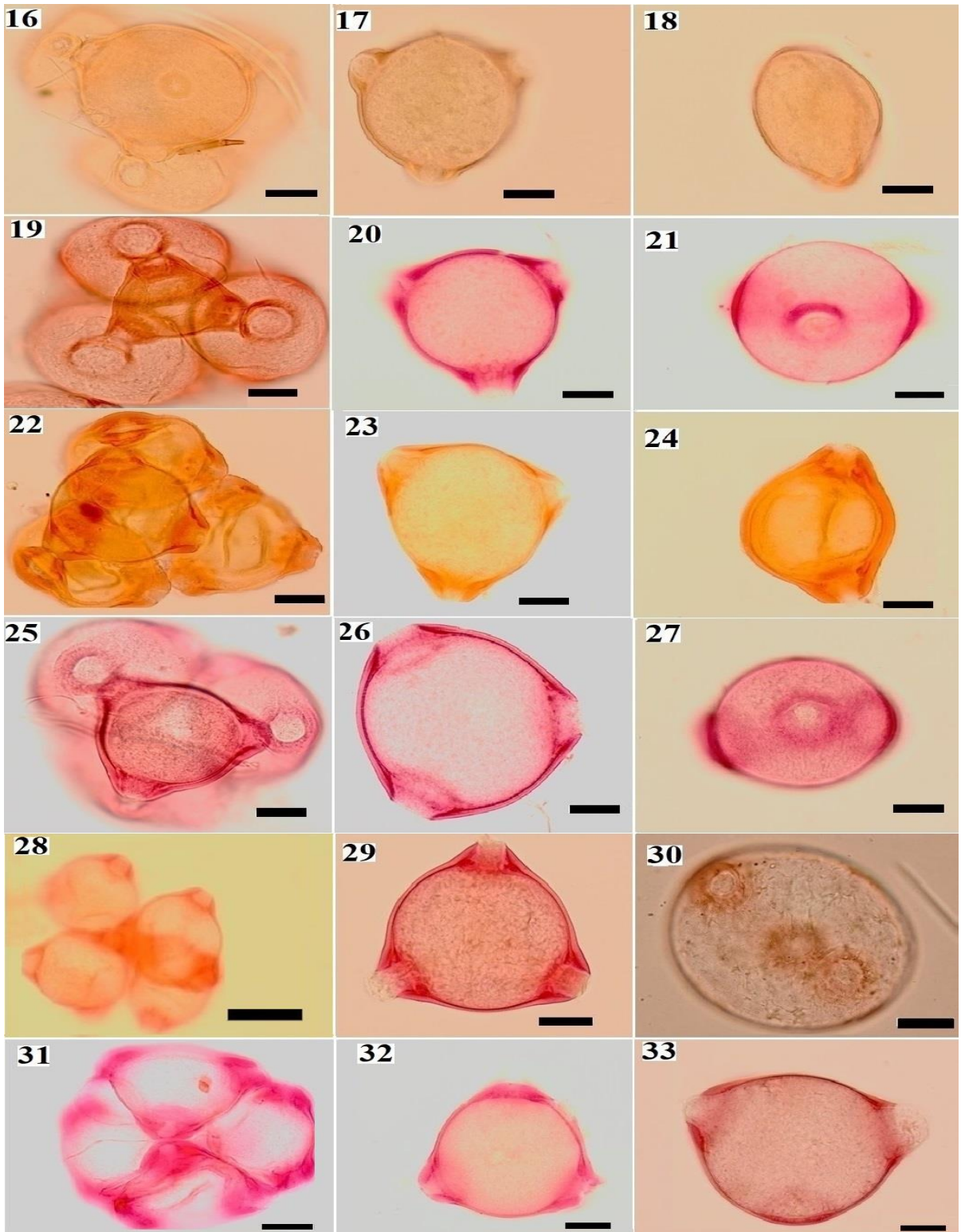


Figure 1. (Continued) (19-21) *E. minutiflorum*, (22-24) *E. confusum*, (25-27) *E. ponticum*, (28-30) *E. gemmascens*, (31-33) *E. anagallidifolium* (Scale bar 20 µm) and (28; Scale bar 50 µm).

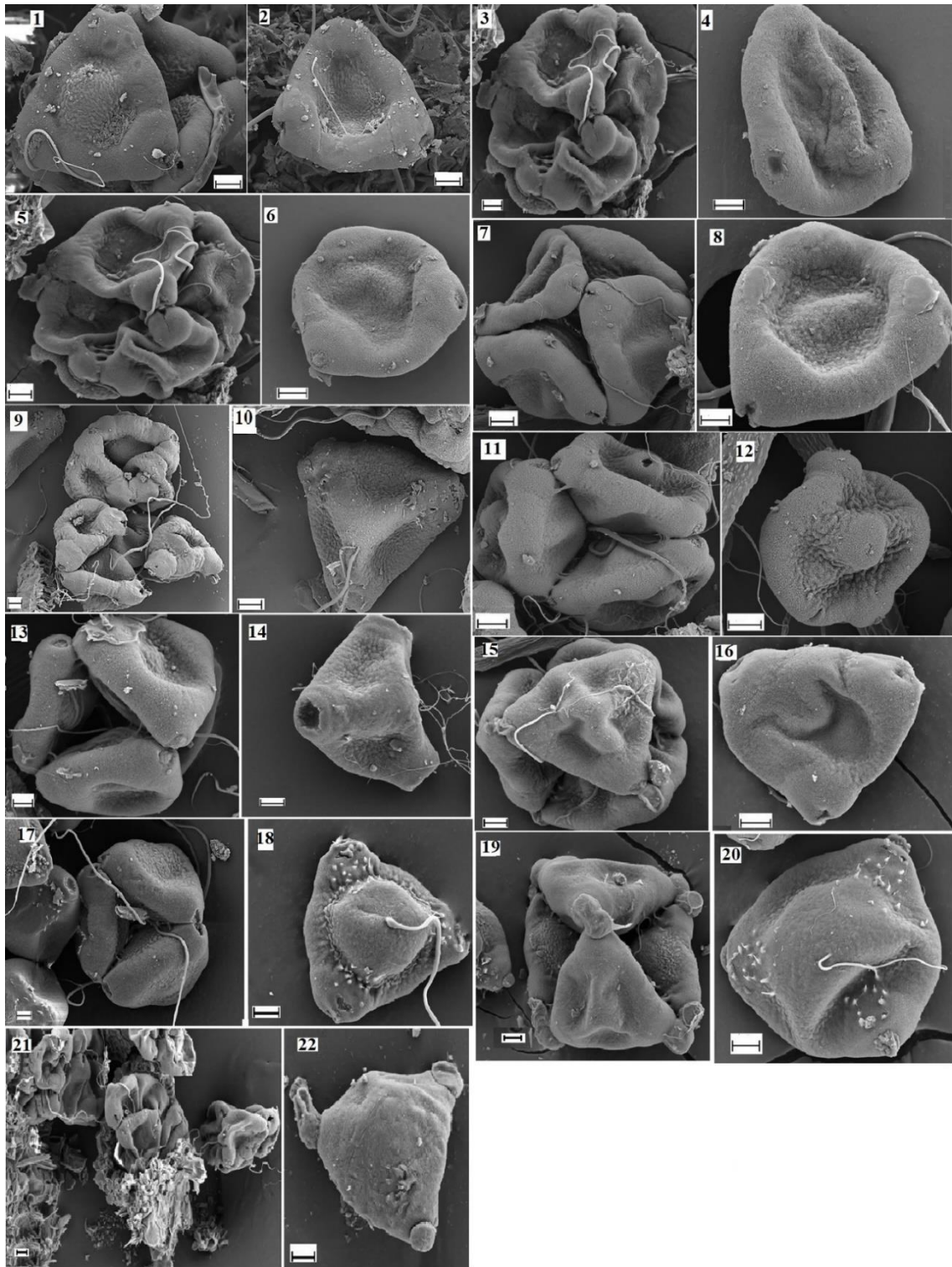


Figure 2. Pollen grains SEM photos of studied taxa: tetrad and general view respectively; **(1-2)** *Epilobium hirsutum*, **(3-4)** *E. parviflorum*, **(5-6)** *E. montanum*, **(7-8)** *E. anatolicum* subsp. *anatolicum*, **(9-10)** *E. anatolicum* subsp. *prionophyllum*, **(11-12)** *E. palustre*, **(13-14)** *E. minutiflorum*, **(15-16)** *E. confusum*, **(17-18)** *E. ponticum*, **(19-20)** *E. gemmascens*, **(21-22)** *E. anagallidifolium* (Scale bar 5 μm).

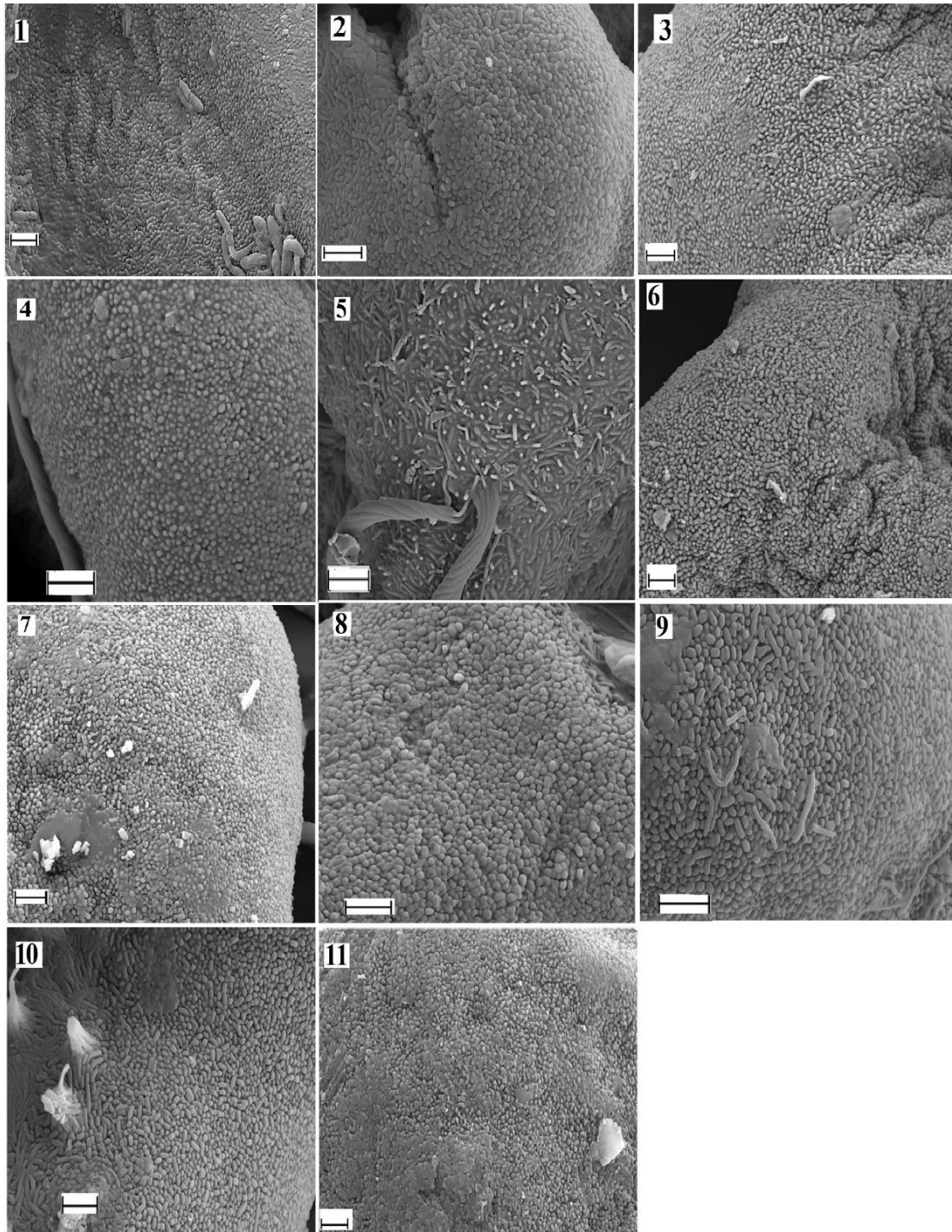


Figure 3. Pollen grains SEM photos of studied taxa: ornamentation; (1) *Epilobium hirsutum*, (2) *E. parviflorum*, (3) *E. montanum*, (4) *E. anatolicum* subsp. *anatolicum*, (5) *E. anatolicum* subsp. *prionophyllum*, (6) *E. palustre*, (7) *E. minutiflorum*, (8) *E. confusum*, (9) *E. ponticum*, (10) *E. gemmascens*, (11) *E. anagallidifolium* (Scale bar 2 μm).

4. Discussion

Pollen grains characteristics of *Epilobium* 11 taxa are summarised in Table 1 and are shown in Figs. 1-3. Pollen grains are tetrad, apertures (angulaperturate) at corners and pollen grains are triangular-obtus, 3-zonoporate (*E. anatolicum* subsp. *anatolicum* 4 % 2-porate, *E. anatolicum* subsp. *prionophyllum* taxa, 3 % 2-porate and 6 % 4-porate and *E. palustre* 6 % 4-porate). *E. montanum*, *E. anatolicum* subsp. *anatolicum*, *E. palustre*, *E. confusum* taxa have suboblate and other taxa have oblate-spheroidal pollen grains shape. *E. palustre* (57.52 μm and 65.42 μm) with the smallest polar axis and equatorial axis has the largest polar axis *E. hirsutum* (87.33 μm and 95.23 μm). The taxon with the smallest pore length and pore width is *E. palustre* (10.81 μm and 11.97 μm). The largest polar axis is *E. hirsutum* (22.03 μm and 22.66 μm). The smallest exine thickness is in *E. montanum* (1.78 μm) and the largest one is in *E. hirsutum* (2.68 μm). The smallest intin thickness is observed in the taxon *E. minutiflorum* (0.39 μm), while the largest thickness is observed in the taxon *E. ponticum* (0.69 μm). *E. ponticum* (4.25 μm) has the smallest annulus thickness, and *E. anatolicum* subsp. *prionophyllum* (6.35 μm) has the greatest thickness. *E. palustre* (14.29 μm) has the shortest annulus height and *E. anatolicum* subsp. *prionophyllum* (23.58 μm) has the largest taxon. The smallest ambi diameter is in *E. palustre* (62.50 μm), and the largest in *E. montanum* (92.74 μm). The shortest mesopodium is in *E. anatolicum* subsp. stated that pollen grains color can also be used for species differentiation from the genus *Clarkia* Pursh in Onagraceae. Skvarla et al. (2008) reported that pollens with tetrahedral tetrad characteristics in Onagraceae are connected to each other by bridges and that the structures called viscin threads formed in tetrahedral pollens. This is also characteristic for *Epilobium* taxa which are the structures that connect the pollen. It was also noted that the palynomorphological characters have taxonomic importance and the importance of more detailed studies in order to obtain more effective results was emphasized in the study.

Punt et al. (2003) determined 7 different pollen types according to micromorphological features such as pollen grain size, aperture type and exine thickness in the Northwest European *Epilobium*. In this genus, three different types of pollen: *Epilobium angustifolium*-type, *E. latifolium*-type and *E. tetragonum*-type were included. The structure of pollen is radial symmetrical, monad and tetrad. Tectum characteristic is pitted-angular, fine granular, rod-like, oblate, suboblate and spheroidal pollen. Their shapes revealed that they have granulate and striate-rugulate ornamentation.

Makbul et al. (2008) revealed that *Epilobium palustre*, *E. algidum* Bieb., *E. ponticum*, *E. confusum*, *E. hirsutum*, *E. montanum* pollen grains are radially symmetrical, isopolar, 3-zonoporate and generally shed in monads, rarely tetrads. Pollen grains shape are suboblate, *E. montanum* (spheroidal). taxa of *Epilobium* are usually tetrahedral tetrads and rarely monads. In the examination carried out on a certain number of

anatolicum (37.15 μm), and the largest one is in *E. montanum* (62.08 μm). Apopodium is seen in the shortest taxon *E. anatolicum* subsp. *anatolicum* (37.15 μm), and the largest in *E. montanum* (60.07 μm). The taxon with the smallest costal thickness is *E. ponticum* (2.45 μm), and the largest one is *E. montanum* (3.35 μm). It was determined that the taxon *E. montanum* has rugulate, *E. anatolicum* subsp. *prionophyllum* and *E. gemmascens* baculate-rugulate, while the other taxa have baculate ornamentation.

Some studies on the palynomorphological features of *Epilobium* genera have been carried out (Chen and Wang, 2001; Punt et al., 2003; Skvarla et al., 2008; Perveen and Qaiser, 2013). The most comprehensive palynological studies on *Epilobium* taxa in our country were made by Makbul et al. (2008); Okur (2019). Makbul et al. (2008) revealed the morphological features of the pollen of *Epilobium* 6 taxon and stated that all species of our country should be studied in order to make more contribution. (Patel et al., 1984; Perveen and Qaiser, 2013; Okur, 2019) determined that the pollen distribution of *Epilobium* is generally tetrahedral tetrad and rarely monad.

Lewis and Lewis (1955) reported that in addition to morphological features such as umb diameter, pore size, exine and ectexin thickness of pollen, the color of fresh pollen is effective in the differentiation of taxa, and Small et al., 1971

The outline is triangular in meridional optical section. Polar axis is 78–67 μm and equatorial axis is 87–94 μm . The aperture membrane is generally psilate and rarely granulate. The exine is tectate. Ornamentation is baculate, *E. ponticum* and *E. confusum* (striate). They stated that palynological characters such as pollen shape and surface ornamentation were effective in distinguishing *Epilobium* taxa.

Perveen and Qaiser (2013) found that the tectum structure of pollen grains belonging to the Onagraceae is an important characteristic. Rugulate-perforate ornamentation was observed in two *Oenothera* species (*O. affinis* Combess. and *O. glazioviana* Michel), which are known for their taxonomic affinity to the genus *Epilobium*, while *Epilobium leiophyllum* Haussn. It has been determined that it has a granulate ornamental structure, as well as *E. angustifolium*, *E. cylindricum* D. Don., *E. hirsutum*, *E. latifolium* L., *E. parviflorum* and *E. palustre* species with scabrate ornamentation.

Erdtman 1960; Punt et al. 2003 revealed that there is a fine connection between the tetrad and monad pollen grains spreads by breaking this connection in the acetolysis process. Skvarla et al. (2008) observed that the pollen of the genus *Epilobium* has both monad and tetrahedral tetrad structures. They revealed that the monad structure, the tetrahedral tetrad structure, was formed as a result of the breakdown of the bridges between pollens and each other during the preparation process. Makbul et al. (2008) observed that the pollens of the 6 taxa, it was determined that the pollen grains in the monad structure supported the stated idea.

As a result of their examination on the pollens of the genus *Epilobium*, Okur et al. (2019), stated that the pollen is monad or tetrahedral or, bilateral, with subisopolar symmetry, 3-porate (trizonoporate). Surface ornamentation is baculate, striate and baculate-rugulate. They determined that the pollen grains in the *Epilobium* section spread in tetrahedral form from the anther and monad in the *Chamaenerion* section. They determined that the pollen shape of the taxa belonging to *Epilobium*, exine ornamentation, and pollen grains leaving the anther, ie spreading (pollen out of the anther monate or tetrahedral) are important in the differentiation of pollen. Okur (2019) determined that *Epilobium* and *Chamaenerion* taxa have baculate, baculate-rugulate, rugulate, baculate-plate ornaments and pollen grains size is between 40.70-53.96 µm. They determined that it may be due to the fact that they have carried out palynomorphological studies on the taxon.

Our results and the data of Okur 2019 were found to be compliant regarding the fact that the taxa are tetrahedral tetrads and ornamentations with trizonoporate (*E. anatolicum* subsp. *anatolicum* 4% 2-porate, *E. anatolicum* subsp. *prionophyllum* taxa, 3% 2-porate and 6% 4-porate and *E. palustre* 6% 4-porate) pollens were observed. On the other hand, using different methods may have caused differences in the polar and equatorial axis, pore length and width, exine and intin thickness, annulus thickness, Amb diameter, mesopodium and apopodium thicknesses.

Epilobium is considered to be a taxonomically difficult genus due to the fact that it contains a large number of taxa with morphological similarities and the high percentage of hybridization within the genus (Krajsek et al., 2006). For this reason, it is emphasized that making a distinction only on morphological characters is not a solution in the differentiation of taxa belonging to the genus and there is a need for various studies that will contribute to this distinction.

In our opinion, revealing the morphological features of the pollen of 11 taxa belonging to *Epilobium* will contribute primarily to plant systematics and other fields of palynology.

Acknowledgements

We wish to thank Curators of Herbaria GAZI and Bitlis Eren University Herbaria, who allowed us to study their *Epilobium* specimens, to Dr. Yüksel AKINAY and Dr. Ihsan Nuri AKKUŞ (Science Application and Research Center, University of Yuzuncu Yil, Van) who is helper to take of electron photographs of pollen grains surface.

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