

## Comparative morphological, anatomical and palynological investigations of the genus *Euphrasia* L. (Orobanchaceae) in Iran

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**Summary:** Comparative morphological, anatomical and palynological studies on six species *Euphrasia* L. (Orobanchaceae) in Iran are presented using plants collected from their type localities and many other populations. *Euphrasia petiolaris* and *E. sevanensis* are reported for the flora of Iran for the first time. In terms of anatomy, the phloem/xylem proportion in vascular bundles of stem and root, presence or absence of collenchyma at the periphery of stem cortex, the number of parenchyma cell layers of stem cortex and the thickness of the vascular bundle in the leaf midrib provide valuable characters in distinguishing species. According to the obtained results, the pollen morphology seems also to be taxonomically valuable. The main shapes observed among investigated taxa were spheroidal, oblate-spheroidal and prolate-spheroidal. The pollen grains were tricolpate and microrugulate, micropilate and microgemmate on exine surface. The relationships between taxa were estimated by analyzing the scored morphological, anatomical and palynological data using the Euclidian distance coefficient and UPGMA clustering method. Keys are provided for identification of the species of *Euphrasia* in Iran based on both morphological and anatomical features.

**Keywords:** anatomy, palynology, *Euphrasia*, Orobanchaceae, taxonomy, flora of Iran

The genus *Euphrasia* comprises about 450 perennial and annual green hemiparasitic species (MABBERLEY 2008). The distribution area ranges from Europe to Asia, the northern parts of America, South America, the mountains of Indonesia, Australia and New Zealand (BARKER 1982). Typical habitats in Iran are located in mountainous areas like in the northern ranges of Alborz (1500–4500 m). Four species are reported for Iran (YEO 1981): *E. hirtella*, *E. juzepczukii*, *E. pectinata*, *E. salisburgensis*. VITEK (1998) pointed out that taxonomic problems of *Euphrasia* have arisen from a wide range of characteristics caused by autogamous breeding systems and patterns of ecological convergences. *Euphrasia* species are facultative hemiparasites and show polymorphism, perhaps as a consequence of local habitat conditions and differences in host species (GUSSAROVA et al. 2008; FRENCH et al. 2005). Experimental cultivation has identified a wide range of possible host species for *Euphrasia* including grasses and legumes (NYLEHN & TOTLAND 1999; MATTHIES 1998; YEO 1964). The genus *Euphrasia* is conventionally assigned to the tribe Rhinanthae of Scrophulariaceae (YEO 1981). In recent years, Rhinanthae have been transferred to Orobanchaceae based on analyses of cpDNA as well as nrDNA ITS sequences which in turn do not support a monophyly of the tribe (OLMSTEAD et al. 2001). A molecular phylogeny of Orobanchaceae based on phytochrome A indicates monophyly of *Euphrasia* and suggests *Odontites*, *Bartsia* and *Parentucellia* as its relatives (BENNETT & MATHEWS 2006).

In Iran this genus comprises annual herbs with sessile and generally opposite upper leaves and spike-like or racemose inflorescences. The corolla is 2-lipped with the upper lip entire or notched, hood-like and the lower lip 3-lobed. *Euphrasia* is known as the taxonomically most problematic genus in Rhinanthae (sensu YEO 1981). The identification of species in the genus is primarily based on leaf and bract characters. There are only few papers dealing with topics of the study

presented here, e.g., on general anatomy of the genus *Odontites* and *Parentucellia* in Iran (SAEIDI-MEHRVARZ 2004) and on leaf anatomy of Turkish Rhinanthae including *E. pectinata* (KAPLAN & İNCEOGLU 2003). İNCEOGLU (1982) published data on pollen morphology of 21 species belonging to 11 genera of the tribe Rhinanthae including *E. pectinata*, *E. juzepczukii* and *E. sevanensis*. LU et al. (2007) also reported on pollen morphology of the tribe Rhinanthae considering *E. pectinata* and *E. hirtella*, too.

This paper provides a detailed description of anatomical features of root, stem and leaf, as well as seed and pollen micro-morphology in *Euphrasia* species growing in Iran, and evaluates the systematic significance of such characteristics.

## Materials and methods

Plants were collected from NW and N Iran in 2010. 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 Guilan University. We used different accessions for each taxon and selected constant characters.

Materials for anatomical studies were fixed in FAA (formaldehyde: acetic acid: alcohol) for 24 h and then preserved in 70% ethanol in the field. Transverse sections of roots, stems and leaves cut by hand were examined. Cross sections were stained with methylene blue and kongo red

Table 1. Collection data of *Euphrasia* examined.

| Species                                     | Collection data                                                                                                                                                                                                                                                                                  |
|---------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>E. hirtella</i> Jord. ex Reut.           | Mazandaran: Garasmasar village, Dozd mountain, 36°49'11" N, 50°34'29" E, 2040 m, Roohi & Roudi 4366; South of Ramsar, between Javaherdeh and Bagh-dasht, 2600 m, Runemark & Massoumi 21733-TARI; Tehran: Darband-Sar, 2700–3400 m, Mozaffarian & Mohammadi 49219-TARI.                           |
| <i>E. juzepczukii</i> Denissova             | Mazandaran: kelardasht, sarchal, Siah gok mountain, 3600–4200 m, Termeh 1531; Azerbaijan: Sabalan, 3470 m, Foroughi & Assadi 13937-TARI; Ardabil, yarabijeh, 38°42'85" N, 46°56'36" E, 2577 m, Roohi & Roudi 4367.                                                                               |
| <i>E. pectinata</i> Ten.                    | Azerbaijan: Arasban protected area, Dughron mountain, 2300 m, Assadi 20971-TARI; Guilan: Asalam to Khalkhal, 1900 m, Wendelbo & Shirdelpour 14905-TARI; Mazandaran: Lar valley, 3000 m, Wendelbo & Assadi 13345-TARI; Tehran: Darband-Sar, 36°01'47" N, 51°28'49" E, 2823 m, Roohi & Roudi 4368. |
| <i>E. petiolaris</i> Wettst.                | Azerbaijan: Ahar, Termeh 13480; Kalybar, 38°50'74" N, 46°58'79" E, 2067 m, Roohi & Roudi 4369.                                                                                                                                                                                                   |
| <i>E. salisburgensis</i> Funk<br>ex Hoppe . | Azerbaijan: Maku to Khoy, South west of Kelisa-Kandi, 2400–2650 m, Assadi & Mozaffarian 30322-TARI; Sahand mountain, Assadi & Mozaffarian 30689-TARI; Ardabil, Alvares, 38°10'24" N, 47°55'47" E, Roohi & Roudi 4370.                                                                            |
| <i>E. sevanensis</i> Juz.                   | Azerbaijan: kalybar, ghaleh babak, 38°50'78" N, 46°58'68" E, 2084 m, Roohi & Roudi 4371; Sabalan, 3470 m, Foroughi & Assadi 13799-TARI; Arasbaran, between Dughron and Makidi, 1800 m, Runemark & Assadi 22027-TARI.                                                                             |

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and covered with glycerol-gelatine (VARDAR 1987). Photographs were taken with an Olympus BH-2 microscope. All measurements and observations were made three or four times from several sections taken from at least two selected specimens.

Terminology of seed-coat and surface sculpturing is according to STEARN (1992).

Palynological investigations were made by both LM (Nikon Optiphot) and SEM. For LM studies pollen slides were prepared according to WODEHOUSE (1935). Pollen grains were gathered from herbarium specimens and the authors' own collections from wild populations. All measurements were determined on at least 30 pollen grains. The pollen grains were also directly placed on prepared stubs and covered with gold for SEM studies. Pollen terminology follows ERDTMAN (1952) and PUNT et al. (2007).

As summarized in Table 2, 28 characters were assessed by a numerical analysis: 11 morphological, 9 anatomical and 8 palynological features were selected. These include quantitative and binary (qualitative) variables. Arithmetic means of each quantitative variable related to anatomy and pollen morphology were separately calculated. Two multivariate analyses were performed using

**Table 2.** Characters used in numerical analysis of the genus *Euphrasia* in Iran. Characters X20–X27 represent the features of pollen grains.

| Symbol | Character                                     |
|--------|-----------------------------------------------|
| X1     | Length of leaf (µm)                           |
| X2     | Width of leaf (µm)                            |
| X3     | Length of bracts (µm)                         |
| X4     | Width of bracts (µm)                          |
| X5     | Length of corolla (µm)                        |
| X6     | Length of capsule (µm)                        |
| X7     | Width of capsule (µm)                         |
| X8     | Length of flowering stem (cm)                 |
| X9     | Number of leaf teeth                          |
| X10    | Number of bract teeth                         |
| X11    | Diameter of midrib (µm)                       |
| X12    | Number of leaf parenchyma                     |
| X13    | Number of cell layers of leaf collenchyma     |
| X14    | Number of cell layers of stem parenchyma      |
| X15    | Number of cell layers of stem collenchyma     |
| X16    | Thickness of stem endodermis (µm)             |
| X17    | Ratio of phloem/xylem of stem vascular bundle |
| X18    | Ratio of phloem/xylem in root                 |
| X19    | Thickness of stem parenchyma (µm)             |
| X20    | P: Polar axis (µm)                            |
| X21    | E: Equatorial axis (µm)                       |
| X22    | P/E ratio                                     |
| X23    | Length of colpi (µm)                          |
| X24    | Width of colpi at middle (µm)                 |
| X25    | Distance between colpi (µm)                   |
| X26    | Distance of colpi to pole (µm)                |
| X27    | Exine (µm)                                    |
| X28    | Length of calyx (µm)                          |

NTSYSpc ver. 2.0 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 Cophenetic coefficient of resemblance for mixed data sets. For PCA, the raw data were used to create a correlation matrix, and two eigenvectors were extracted, providing two axes on to which the raw data were projected to give a two-dimensional plot of the taxa and characters.

## Results

### Morphological description of the genus *Euphrasia* in Iran

Annual hemiparasitic herbs, stems pubescent, branched, with extended internodes, flowering stem 3–39 cm long. Stem and branches rarely eglandular and glandular hairs. Leaves subsessile, crenate or dentate, opposite or subopposite (on upper parts), 2–16 × 1–11 mm. Bracts similar to leaves, but slightly larger, 3–19 × 2–14 mm, sessile or shortly petiolate, 1–2 mm. Flowers single in axils of leaf-like bracts. Calyx 3–9 mm, 4-toothed, densely glandular. Corolla 4–10 mm, bilabiate, white or lilac, distinctly streaked with blackish violet, lower lip flat, trilobate with emarginate lobes, upper lip cucullate, shortly bilobed. Stamens 4, didynamous; filaments curved, inserted in corolla throat; anther loculi proximally spurred, posterior spurs of posterior anthers are the longest. Stigma capitate. Ovary laterally compressed; capsule 3–8 × 2–4 mm, dehiscent in upper half. Seeds fusiform, furrowed.

#### Morphological key of Iranian *Euphrasia* species

- 1 Bracts more than 1.5 × as long as broad, linear to lanceolate; teeth of bracts and leaves at least the 2 distal pairs distant from one another; capsule ciliate only at the upper part ..... *E. salisburgensis*
- Bracts not more than 1.5 × as long as broad, orbicular-ovate to ovate; teeth of bracts and leaves contiguous; capsule ciliate all over ..... 2
- 2 Leaves and bracts covered by glandular hairs consisting of stalks with more than 2 cells ..... *E. hirtella*
- Leaves and bracts covered by eglandular hairs ..... 3
- 3 Corolla 4.5–6 mm; leaves with 3–5 pairs of teeth, mostly acute ..... 4
- Corolla 6.5 mm or more; leaves with 5 or more pairs of teeth, obtuse or acute ..... 5
- 4 Bracts obovate-lanceolate or obdeltoid, sometimes with a second pair of lateral teeth; corolla without distinct stripes ..... *E. juzepczukii*
- Bracts ovate to suborbicular, with 3–4 pairs of lateral teeth; corolla with dark violet stripes ..... *E. sevanensis*
- 5 Flowers with a pedicel 2–3 mm; leaves and bracts covered by glandular hairs .... *E. petiolaris*
- Flowers sessile; leaves and bracts covered by eglandular hairs ..... *E. pectinata*

#### New records

The genus *Euphrasia* is represented by 4 species in Iran, namely *E. hirtella*, *E. juzepczukii*, *E. pectinata*, *E. salisburgensis* (YEO 1981). *E. petiolaris* and *E. sevanensis* are reported for the flora of Iran for the first time. On account of this study, these two species could be added to the flora

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of Iran. The type specimens of *E. petiolaris* and *E. sevanensis* have already been known from Armenia and Lazistan, located in the Republic of Georgia (SCHISCHKIN & BOBROV 1997). They are deposited at the herbaria in St. Petersburg [LE!] and Vienna [W!], respectively. Occurrence of *E. petiolaris* has been reported from NE Turkey by YEO (1978). SORGER & BÜCHNER (1983) recorded *E. sevanensis* in NE and N of Turkey at altitudes of 1500–2700 m. *E. petiolaris* and *E. sevanensis* were collected from NW Iran at altitudes of 1800–4200 m (Fig. 1). From the chorological point of view, these species belong to Hyrcano-Euxine elements.

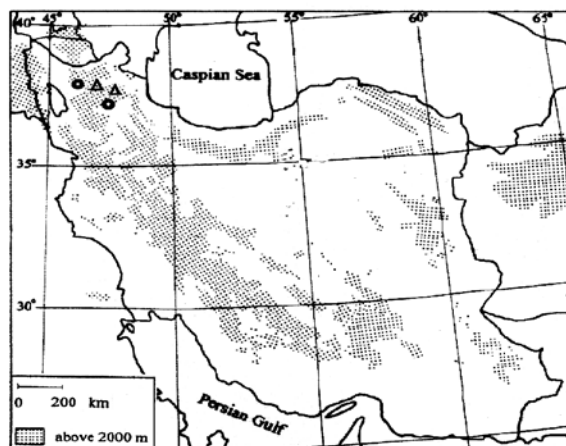


Figure 1. Distribution of *E. petiolaris* ( $\Delta$ ) and *E. sevanensis* (O) in Iran.

## Anatomical features

### Root

In cross section the root is circular in all studied taxa. The epidermis consists of a single layer of rectangular or orbicular cells and is surrounded by a cuticle layer. Cortex parenchyma below the epidermis is thin-walled, 2 cell layers thick, with small intercellular space. The endodermis consists of one layer of rectangular cells. At the basal part, roots show secondary growth. The vascular system consists of numerous secondary vascular bundles situated very close to each other, so that the intervacular regions are difficult to distinguish. The vascular tissue forms a continuous complete cylinder (Fig. 2: 1–6).

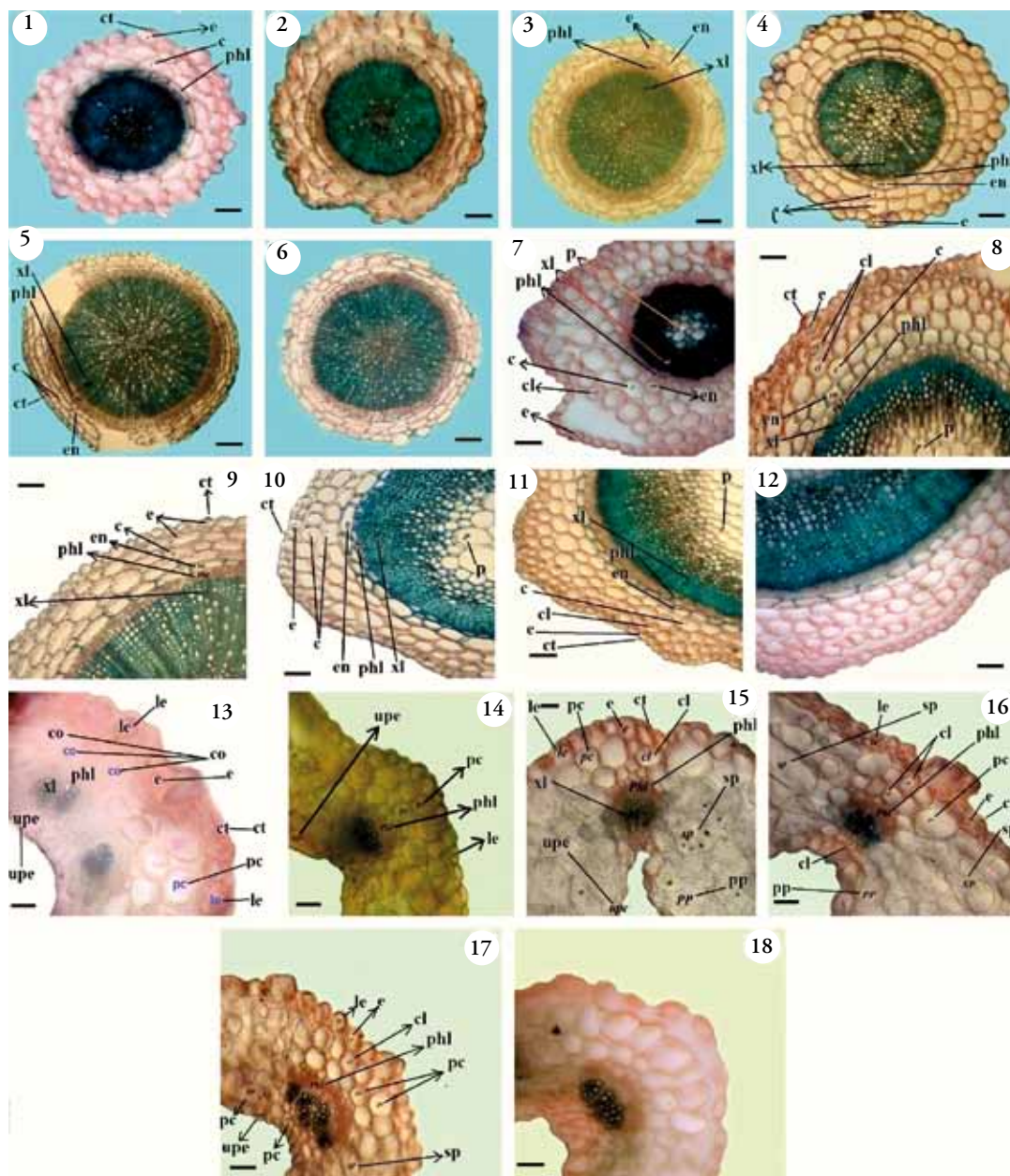
### Stem

The transverse section of the stem is quadrangular shaped. The epidermis consists of a single layer of rectangular or orbicular cells and is surrounded by a cuticle layer. There are glandular or eglandular hairs on the epidermis. Below the epidermis there is a 1–2 layers thick lamellar collenchyma or it is absent. Cortex parenchyma is thin-walled, 1–3 cell layers thick, usually elliptic, with intercellular space. The endodermis consists of a single layer of rectangular or ovoid cells. The phloem has irregular cells. The stem shows a secondary growth at its basal parts. The pith is wide and consists of hexagonal or orbicular parenchymatous cells (Fig. 2: 7–12).

### Leaf

The leaves are dorsiventral. In transverse section, the upper and lower epidermises comprise uniseriate, oval and rectangular cells. The cuticle is well developed both on abaxial and adaxial

surfaces. Sparse indumentum appears on the surface, made of two types of non-glandular and glandular trichomes. The leaves are amphistomatic, with stomata slightly raised above the epidermis level and more numerous on the lower side of the leaf. The mesophyll is differentiated into palisade and spongy parenchyma. The palisade parenchyma is 2 layers thick. The spongy parenchyma consists of 3–4 layers of irregular form, it is relatively compact and has small



**Figure 2.** Transverse sections of roots: 1: *E. juzepczukii*, 2: *E. petiolaris*, 3: *E. sevanensis*, 4: *E. salisburgensis*, 5: *E. hirtella*, 6: *E. pectinata*. Transverse sections of stem: 7: *E. juzepczukii*, 8: *E. petiolaris*, 9: *E. sevanensis*, 10: *E. salisburgensis*, 11: *E. hirtella*, 12: *E. pectinata*. Transverse sections of leaves: 13: *E. juzepczukii*, 14: *E. petiolaris*, 15: *E. sevanensis*, 16: *E. salisburgensis*, 17: *E. hirtella*, 18: *E. pectinata*. – Abbreviations: ct – cuticle; e – epidermis; c – cortex; cl – collenchyma; en – endodermis; phl – phloem; xl – xylem; p – pith; le – lower epidermis; upe – upper epidermis; pc – cortex parenchyma; pp – palisade parenchyma; sp – spongy parenchyma. Scale bars: 1–12 = 50 µm; 13–18 = 40 µm.

Morphology, anatomy and palynology of *Euphrasia* in Iran**Table 3.** Anatomical characters of Iranian *Euphrasia* species.

| Species<br>Character                      | <i>E. hirtella</i>       | <i>E. juzepczukii</i>      | <i>E. pectinata</i>       | <i>E. salisburgensis</i> | <i>E. sevanensis</i>     |
|-------------------------------------------|--------------------------|----------------------------|---------------------------|--------------------------|--------------------------|
| Thickness of root parenchyma (µm) *       | (30)<br>35±5<br>(40)     | (60)<br>65±5<br>(70)       | (20)<br>22.5±2.5<br>(25)  | (63)<br>70.5±7.5<br>(78) | (35)<br>42.5±7.5<br>(50) |
| Thickness of root endodermis (µm) *       | (10)<br>12.5±2.5<br>(15) | (10)<br>12.5±2.5<br>(15)   | (8)<br>10±2<br>(12)       | (15)<br>17.5±2.5<br>(20) | (12)<br>14.5±2.5<br>(17) |
| Ratio of phloem/xylem in root             | 19%                      | 11%                        | 15%                       | 3%                       | 19%                      |
| Thickness of stem epidermis (µm) *        | (10)<br>12.5±2.5<br>(15) | (15)<br>17.5±2.5<br>(20)   | (15)<br>17.5±2.5<br>(20)  | (15)<br>17±2<br>(19)     | (10)<br>15±5<br>(20)     |
| Number of cell layers of stem collenchyma | 1                        | 1                          | 0                         | 0                        | 0                        |
| Number of cell layers of stem parenchyma  | 2                        | 1                          | 3                         | 2                        | 3                        |
| Thickness of stem endodermis (µm) *       | (10)<br>15±5<br>(20)     | (20)<br>30±10<br>(40)      | (17)<br>21±4<br>(25)      | (20)<br>25±5<br>(30)     | (12)<br>18.5±6.5<br>(25) |
| Ratio of phloem/xylem in stem             | 22%                      | 40%                        | 12%                       | 3%                       | 43%                      |
| Thickness of midrib (µm) *                | (85)<br>103±18<br>(121)  | (87)<br>99.5±12.5<br>(112) | (68)<br>78.5±10.5<br>(89) | (63)<br>66.5±3.5<br>(70) | (78)<br>81.5±3.5<br>(85) |
| Number of leaf parenchyma                 | 2                        | 2                          | 2                         | 0                        | 0                        |
| Number of cell layers of leaf collenchyma | 1                        | 1                          | 1                         | 3                        | 3                        |

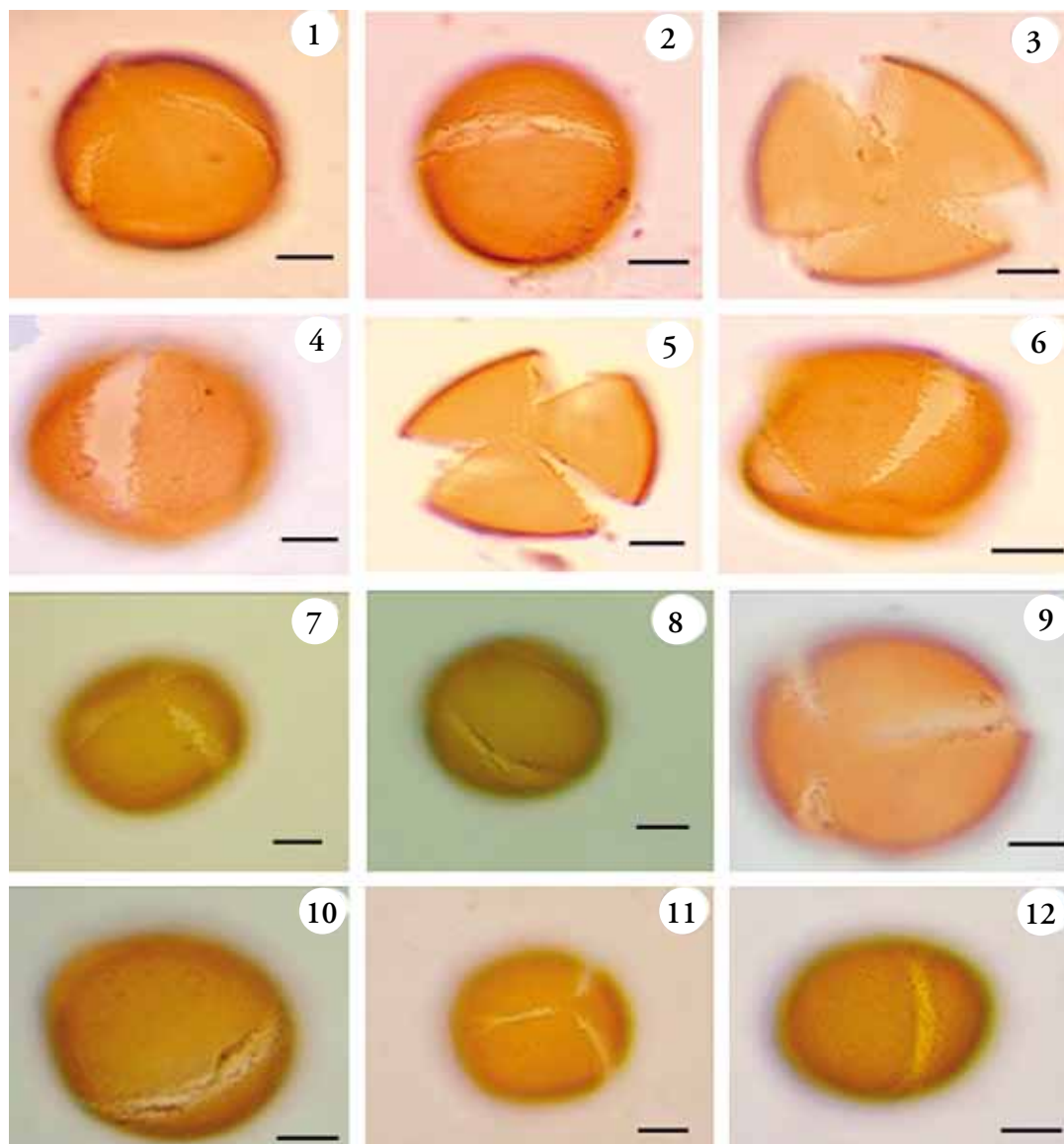
\*Numbers are (Min), mean value ± standard deviation, (Max).

intercellular spaces. Midrib is semicircular shaped and has 1–3-layered collenchymas. Arc-shaped vascular bundles are surrounded by orbicular parenchymatous cells. The xylem faces towards the upper surface, while the phloem faces the lower epidermis (Fig. 2: 13–18). An anatomical comparison of 6 *Euphrasia* species in Iran is presented in Table 3.

Anatomical key of Iranian *Euphrasia* species

- 1 Collenchyma in stem cortex present ..... 2
- Collenchyma in stem cortex absent ..... 4
- 2 Parenchyma of stem cortex 1 layer thick; trichomes 1- or 2-celled ..... 3
- Parenchyma of stem cortex 2 layers thick; trichomes with more than 2 cells ..... *E. hirtella*
- 3 Collenchyma of stem cortex 2 layers thick; leaf collenchyma 2 layers thick; phloem/  
xylem ratio in root > 14% ..... *E. petiolaris*

- Collenchyma of stem cortex 1 layer thick; phloem/xylem ratio in root < 14%  
..... *E. juzepczukii*
- 4 Parenchyma of cortex stem 3 layers thick; phloem/xylem ratio in root > 14% ..... 5
- Parenchyma of cortex stem 2 layers thick; phloem/xylem ratio in root < 14%  
..... *E. salisburgensis*
- 5 Collenchyma of leaf 1 layer thick; phloem/xylem ratio in stem > 17% ..... *E. pectinata*
- Collenchyma of leaf 3 layers thick; phloem/xylem ratio in stem < 17%  
..... *E. sevanensis*

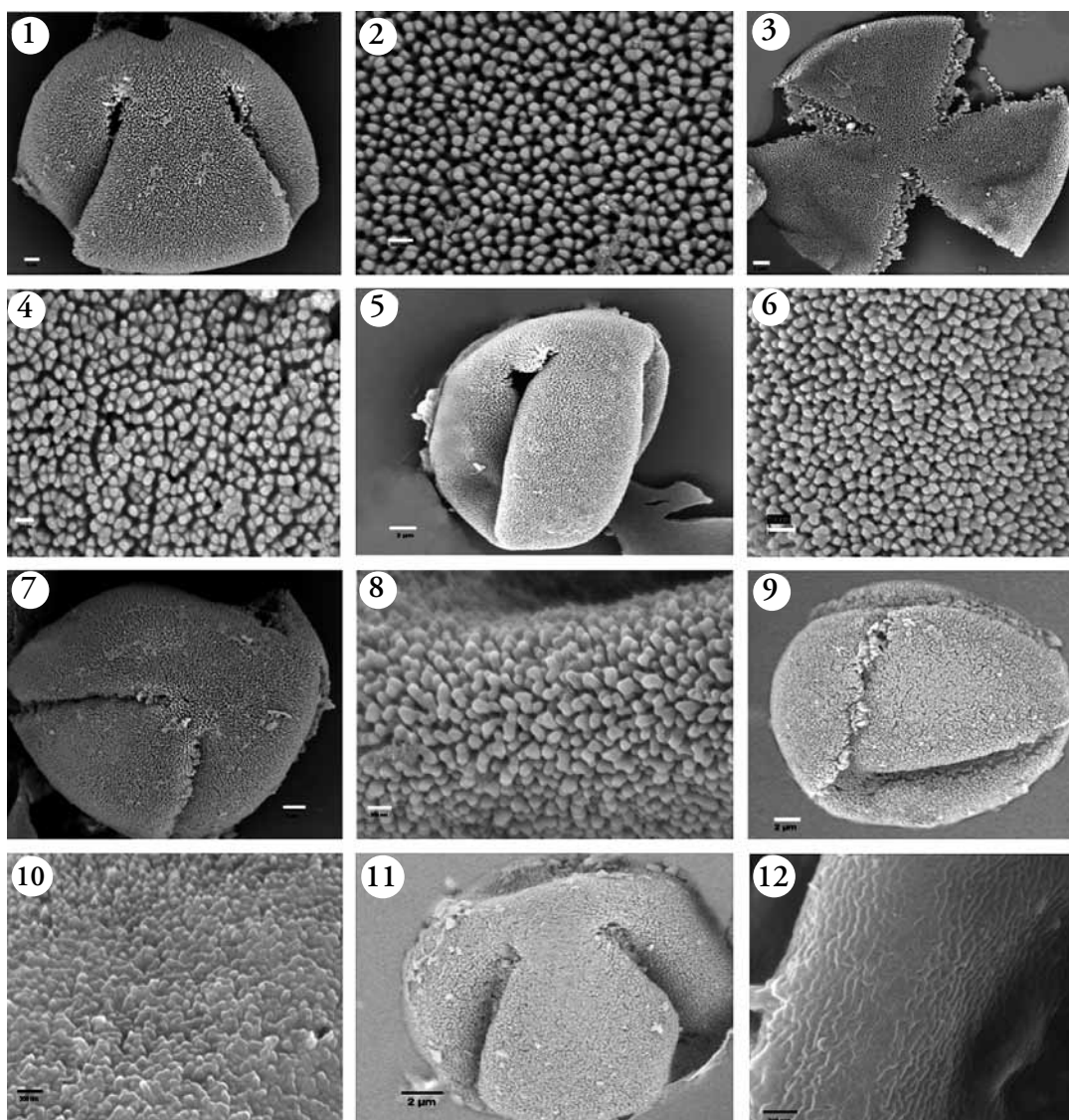


**Figure 3.** LM micrographs of pollen grains of Iranian *Euphrasia* species: 1, 2 – *E. pectinata*; 3, 4 – *E. hirtella*; 5, 6 – *E. salisburgensis*; 7, 8 – *E. petiolaris*; 9, 10 – *E. sevanensis*; 11, 12 – *E. juzepczukii*. Polar view (1, 3, 5, 7, 9, 11); equatorial view (2, 4, 6, 8, 10, 12). Scale bars = 10  $\mu$ m.

## Pollen morphology

Pollen grains of examined species are shown in Figs 3–4. The palynological characteristics of the examined specimens are given in Table 4. The pollen grains of all examined species of *Euphrasia* are tricolpate, radially symmetrical and isopolar. The P/E ratio varies from 0.7 to 1.2. So, the shapes of grains are spheroidal, oblate-spheroidal and prolate-spheroidal. Exine is tectate and 1.75–3 µm thick. Three pollen types are distinguished regarding exine sculpturing: microgemmate, microrugulate and micropilate.

*Euphrasia pectinata*: Pollen grains oblate-spheroidal (Fig. 3: 1–2), 33–37 × 32.9–39.9 µm, ornamentation microgemmate, sculptural elements in rounded or polygonal patterns, individual microgemmae laterally fused (Fig. 4: 1–2). Exine tectate and 2–2.5 µm thick.



**Figure 4.** SEM micrographs of pollen grains of Iranian *Euphrasia* species: 1, 2 – *E. pectinata*; 3, 4 – *E. hirtella*; 5, 6 – *E. salisburgensis*; 7, 8 – *E. sevanensis*; 9, 10 – *E. petiolaris*; 11, 12 – *E. juzepczukii*. Scale bars: 3, 7 = 3 µm; 1, 5, 9, 11 = 2 µm; 2, 4, 6 = 0.5 µm; 8, 10, 12 = 0.3 µm.

**Table 4.** Pollen morphological characters of Iranian *Euphrasia* species. Abbreviations: P – polar axis; E – equatorial axis; CL – length of colpi; CW – width of colpi; M – distance between colpi; A – distance colpi to pole; EX – exine thickness; EO – exine ornamentation.

| Species<br>Characters | <i>E. hirtella</i>           | <i>E. juzepczukii</i>        | <i>E. pectinata</i>          | <i>E. petiolaris</i>         | <i>E. salisburgensis</i>     | <i>E. sevanensis</i>         |
|-----------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
| P (μm) *              | (26.5)<br>30.5±4<br>(34.5)   | (34.7)<br>35.9±1.2<br>(37.1) | (33)<br>35±2<br>(37)         | (29.1)<br>32.3±3.2<br>(35.5) | (31.7)<br>34.9±3.2<br>(38.1) | (37.9)<br>40.1±2.2<br>(42.3) |
| E (μm) *              | (28.7)<br>31.7±3<br>(34.7)   | (28.8)<br>34.3±5.5<br>(39.8) | (32.9)<br>36.4±3.5<br>(39.9) | (31.8)<br>36±4.2<br>(40.2)   | (31)<br>36.5±5.5<br>(42)     | (35.9)<br>38±2.1<br>(40.1)   |
| P/E*                  | (0.7)<br>0.9±0.2<br>(1.1)    | (1)<br>1.1±0.1<br>(1.2)      | (0.8)<br>0.9±0.1<br>(1)      | (0.7)<br>0.8±0.1<br>(0.9)    | (0.7)<br>0.9±0.2<br>(1.1)    | (0.9)<br>1±0.1<br>(1.1)      |
| CL (μm) *             | (19.2)<br>24.2±5<br>(29.2)   | (25.1)<br>27.1±2<br>(29.1)   | (29)<br>31.5±2.5<br>(34)     | (24)<br>25.5±1.5<br>(27)     | (23)<br>27.5±4.5<br>(32)     | (32.9)<br>35.2±2.3<br>(37.5) |
| CW (μm) *             | (4.7)<br>7.8±3.1<br>(10.9)   | (1.7)<br>3.9±2.2<br>(6.1)    | (3.7)<br>4.9±1.2<br>(6.1)    | (1.9)<br>4.1±2.2<br>(6.3)    | (5.9)<br>8.1±2.2<br>(10.3)   | (3.4)<br>4.2±0.8<br>(5)      |
| M (μm) *              | (22.2)<br>25.7±3.5<br>(29.2) | (20.4)<br>25.5±5.1<br>(30.6) | (24.2)<br>26.6±2.4<br>(29)   | (24)<br>29±5<br>(34)         | (25.1)<br>26.8±1.7<br>(28.5) | (29.8)<br>30.6±0.8<br>(31.4) |
| A (μm) *              | (5.3)<br>6.5±1.2<br>(7.7)    | (4.6)<br>6.3±1.7<br>(8)      | (4.4)<br>5.8±1.4<br>(7.2)    | (4.2)<br>6.4±2.2<br>(8.6)    | (6.4)<br>8.2±1.8<br>(10)     | (5.5)<br>6.6±1.1<br>(7.7)    |
| EX (μm) *             | (2)<br>2.25±0.25<br>(2.5)    | (2)<br>2.25±0.25<br>(2.5)    | (2)<br>2.25±0.25<br>(2.5)    | (1.75)<br>1.87±0.13<br>(2)   | (2)<br>2.5±0.5<br>(3)        | (2.5)<br>2.75±0.25<br>(3)    |
| EO                    | m-gemmate                    | m-rugulate                   | m-gemmate                    | m-pilate                     | m-gemmate                    | m-pilate                     |

\*Numbers are (Min), mean value ± standard deviation, (Max).

*Euphrasia hirtella*: Pollen grains oblate-spheroidal (Fig. 3: 3–4), 26.5–34.5 × 28.7–34.7 μm, ornamentation microgemmate, sculptural elements in rounded or polygonal patterns, individual microgemmae separated (Fig. 4: 3–4). Exine tectate and 2–2.5 μm thick.

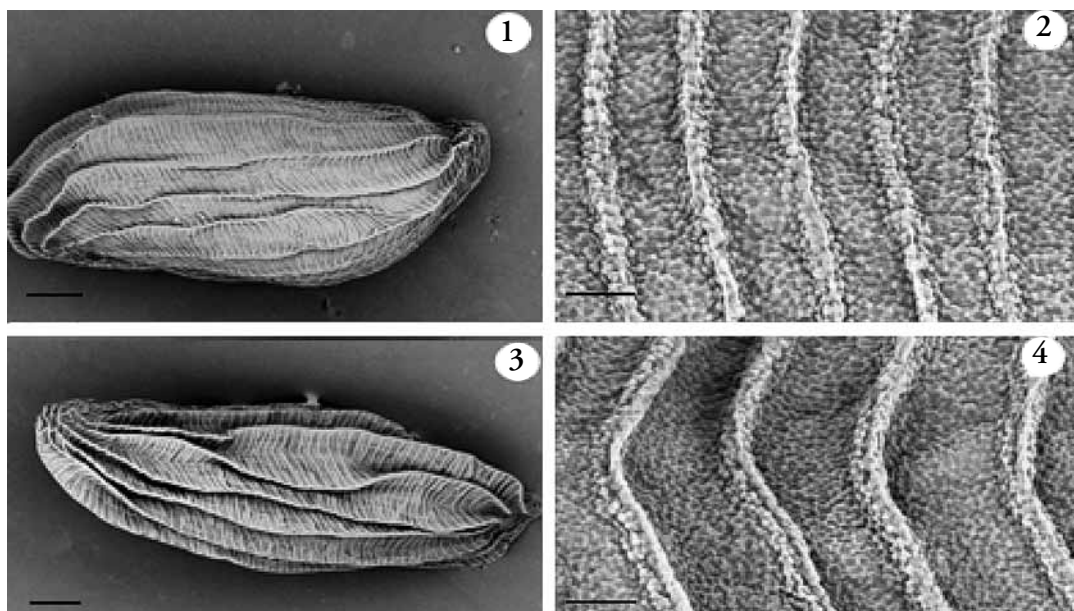
*Euphrasia salisburgensis*: Pollen grains oblate-spheroidal (Fig. 3: 5–6), 31.7–38.1 × 31–42 μm, ornamentation microgemmate, sculptural elements in rounded or polygonal patterns, individual microgemmae laterally fused (Fig. 4: 5–6). Exine tectate and 2–3 μm thick.

*Euphrasia petiolaris*: Pollen grains oblate-spheroidal (Fig. 3: 7–8), 29.1–35.5 × 31.8–40.2 μm, ornamentation micropilate (Fig. 4: 7–8). Exine tectate and 1.75–2 μm thick.

*Euphrasia sevanensis*: Pollen grains spheroidal (Fig. 3: 9–10), 37.9–42.3 × 35.9–40.1 μm, ornamentation micropilate (Fig. 4: 9–10). Exine tectate and 2.5–3 μm thick.

*Euphrasia juzepczukii*: Pollen grains prolate-spheroidal (Fig. 3: 11–12), 34.7–37.1 × 28.8–39.8 μm, ornamentation microrugulate (Fig. 4: 11–12). Exine tectate and 2–2.5 μm thick.

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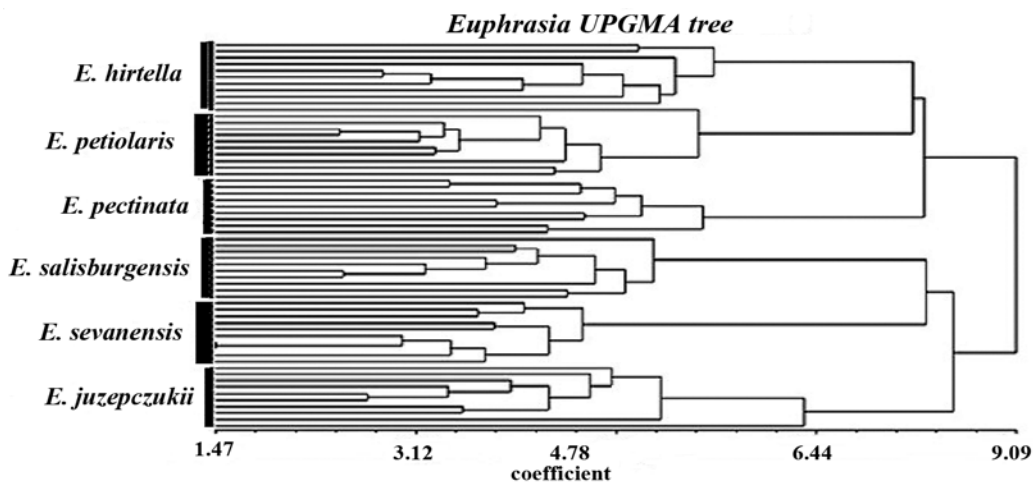
**Figure 5.** SEM micrographs of seed coat: 1, 2 – *E. petiolaris*; 3, 4 – *E. sevanensis*. Whole seed, dorsal view (1, 3), surface ornamentation (2, 4). Scale bars: 1, 3 = 100  $\mu$ m; 2, 4 = 10  $\mu$ m.

### Seed morphology

Seeds of the examined taxa of the genus *Euphrasia* are ellipsoid to prolate, narrowed at both ends, biconvex, both sides with 5–8 narrow, longitudinal white bows, ventral side flat or convex with 4–8 white bows, one lateral edge with a broader bow, 0.9–1  $\times$  0.5–0.6 mm. Surface of the seed corpus fine verrucose. The seed coats of new records, e.g. *E. petiolaris* and *E. sevanensis*, are shown in Fig. 5.

### Numerical analysis

The dendrogram resulting from UPGMA based on 28 variables is presented in Fig. 6. The six examined *Euphrasia* species fall into two major clusters each containing three species. The first



**Figure 6.** Phenogram of the 6 OTUs studied. UPGMA clustering.

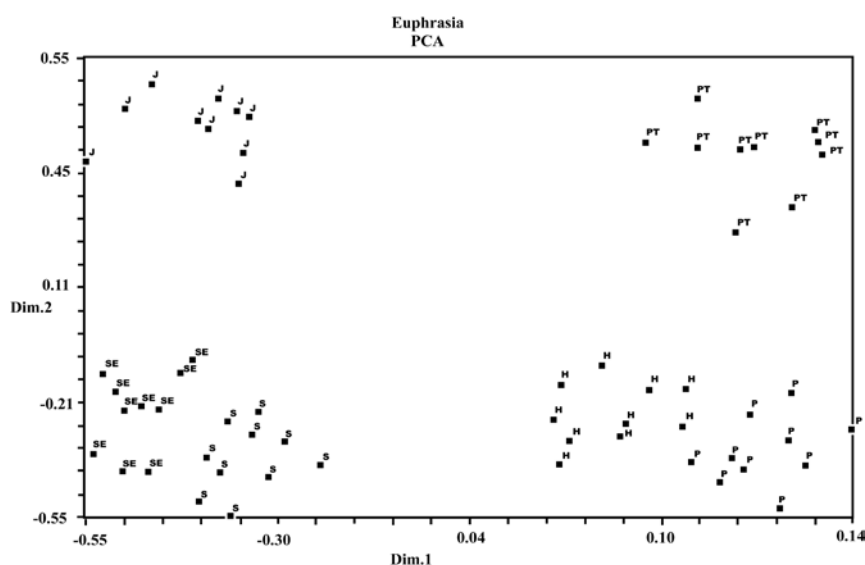
cluster includes *E. hirtella*, *E. petiolaris* and *E. pectinata*, which are linked to each other at 7.3% similarity level, and the second one includes *E. salisburgensis*, *E. sevanensis* and *E. juzepczukii*, which are linked to each other at 7.5% similarity level. Our dendrogram has a cophenetic correlation of 0.84, suggesting that it provides an accurate representation of the resemblances and indicating the best fit between the phenogram and the original distance matrix.

The PCA analysis confirmed the existence of six taxa in the genus as suggested by the cluster analysis (Fig. 7). 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. The two PCA axes were responsible for 62% of variations. Most of the traits are not important in explaining total variation, so only the PCA results for ten characters are given in Fig. 8. Principal component analysis showed that the anatomical characters are more important than the palynological ones in explaining the total variation among the examined taxa.

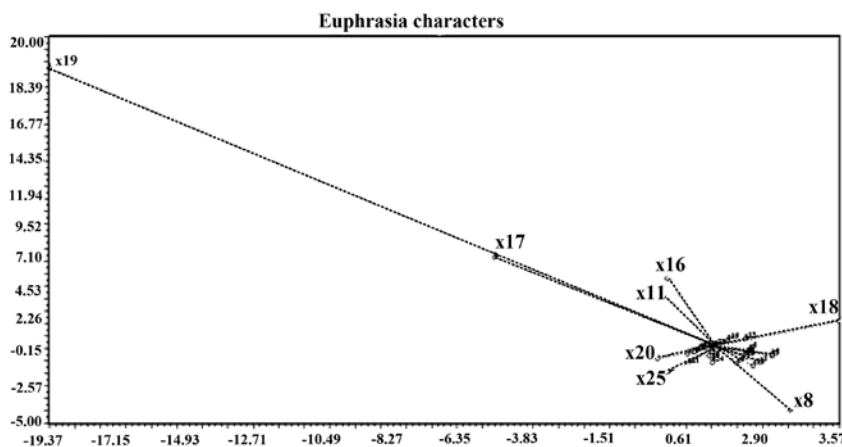
## Discussion

This study provides useful anatomical and palynological information and additional perspectives to the systematics of the examined *Euphrasia* taxa. This is the first anatomical and palynological study of this genus in Iran. We used different accessions for each taxon and selected characters that were constant in them. Among the morphological traits, leaf and corolla characters are important for the separation of taxa. For example, the bracts are 1.5 times as long as broad in *E. salisburgensis* while in other species they are not much longer than broad. Furthermore, the corolla is longer than 6.5 mm in *E. petiolaris* and *E. pectinata* while shorter than 6 mm in *E. sevanensis* and *E. juzepczukii*.

No significant differences in seed morphology have been observed among the *Euphrasia* species distributed in Iran. There are only minor variations regarding the size that could probably be influenced by ecological conditions. Thus, seed morphology of this genus is very homogenous. The common type of seed surface is verrucose.



**Figure 7.** Plot of principal components (PC) for Iranian *Euphrasia* species. OTUs identified are indicated as symbols: PT – *E. petiolaris*; P – *E. pectinata*; H – *E. hirtella*; SE – *E. sevanensis*; S – *E. salisburgensis*; J – *E. juzepczukii*.

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**Figure 8.** Principal component analysis of 6 taxa and 8 variables (X8, X11, X16, X17, X18, X19, X20 and X25). For explanations of variable numbers see Table 2.

Root anatomy is similar in the examined taxa, but the ratio of phloem/xylem varies among the taxa. This ratio in *E. salisburgensis* (3%) obviously differs from other examined species (Fig. 2: 4).

Stem anatomy is also constant in the examined taxa, but the number of collenchyma and parenchyma cell layers varies among the taxa. The presence and number of collenchyma layers in the stem cortex is of a considerable taxonomic value.

Leaf anatomy provides several systematically significant characters. We found that the presence and number of collenchyma layers are particularly significant characters in the examined taxa.

The palynological data presented here reinforce the close relationship among *Euphrasia* species distributed in Iran. Our results are in accordance with İNCEOGLU (1982), who reported that pollen grains of *Euphrasia* are tricolpate, radially symmetrical and isopolar. Three types of exine microsculpturing were observed. The microrugulate exine ornamentation of *E. juzepczukii* differs from other taxa. *E. petiolaris* is palynologically very similar to *E. sevanensis* and its exine is micropilate, while exine ornamentation of *E. hirtella*, *E. pectinata* and *E. salisburgensis* are microgemmate.

Moreover, the P/E ratio varies among the taxa. This ratio is 0.8–1 µm, 0.9–1.1 µm and 1–1.2 µm in *E. pectinata*, *E. sevanensis* and *E. juzepczukii*, respectively. In some *Euphrasia* species, LU et al. (2007) observed suboblate to prolate-spheroidal pollen grains and retipilate ornamentation. This is very interesting because HESSE et al. (2009: 48) refuse the term ‘retipilate’ arguing that no examples of real retipilate ornamentation are known. In our study we found oblate-spheroidal pollen grains along with microgemmate ornamentation in *E. hirtella* and *E. pectinata*. Therefore, the differences in exine ornamentation and P/E ratio of pollen grains seem to be useful in taxonomic delimitation of species in *Euphrasia*.

In cluster analysis a cross line in the dendrogram resulting from UPGMA at 7.1 similarity level divided the OTUs in six groups representing the six recognized species of the genus in Iran. According to PANKHURST (1991) the highest cophenetic correlation represents less distortion and a better classification and is thus an indication that clustering is a good representation of the original dataset. Results of the principal component analysis confirmed the patterns of variation among the accessions studied. The biological implication of principal component analysis can

be quantified from the contribution of the different variables to each principal component. The mean contributions of length of flowering stem, diameter of midrib, thickness of stem endodermis, phloem/xylem ratio in root, thickness of stem parenchyma, phloem/xylem ratio in stem, leaf midrib width and length of colpi were among the highest in the principal axes. This observation suggests that these characters account for most of the variations in *Euphrasia*. The most important character was the thickness of stem parenchyma. Moreover, the palynological features are more important than the anatomical and morphological ones in explaining variation among the examined *Euphrasia* taxa. The results revealed that further molecular studies are essential to solve the problems of relationships and taxonomy of this genus.

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