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Morphology, anatomy and karyology of endangered Turkish endemic *Physoptychis haussknechtii* Bornm. (Brassicaceae) from Central Anatolia

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Abstract – In this study, the morphology, anatomy, pollen morphology, leaf and seed micromorphology and karyology of the Turkish endangered endemic *Physoptychis haussknechtii* were investigated in detail for the first time. The species was studied in terms of detailed morphology. Basal leaves are narrowly spatulate and cauline leaves are linear to oblanceolate; both leaves are covered with flattened stalked five-armed trichomes. Raceme length was found to be up to 6 cm and 9 cm in the flowering and fruiting stages, respectively. Sepals are linear-oblong, rarely elliptic with obtuse to subacute apex. Petals are spatulate with emarginate apex. Some morphological characteristics such as dimensions of cauline leaves and ovary and length of pistil were measured for the first time. In an anatomical study, root, stem, basal leaf and cauline leaf were examined. The anatomical results showed that the plants have secondary growth in roots, primary growth in stems, amphistomatic and equifacial basal and cauline leaves with anisocytic stomata. Pollen grains are radial symmetrical, isopolar and tricolpate. The ratio of polar axis to equatorial diameter is 1.16, and pollen shape is subprolate. Exine sculpturing is reticulate under light microscope and scanning electron microscope. Seeds are obovate-suborbicular, compressed and pale brown. The chromosome number of *P. haussknechtii* was defined to be 2n=16. The karyotype formula of *P. haussknechtii* consists of five metacentric chromosome pairs and three submetacentric chromosome pairs.

Keywords: anatomy, Cruciferae, endemic, karyology, morphology, *Physoptychis haussknechtii*, pollen, Turkey

Introduction

The Brassicaceae family is represented by 321 genera and about 3660 species in the world, and with these numbers it is known as a huge dicot family (Al-Shehbaz 2012). The major distribution centers of the family are the Mediterranean, Irano-Turanian and Saharo-Sindian regions (Hedge 1976). Turkey is one of the richest countries in this respect, with 653 native species belonging to 61 genera of the Brassicaceae family (Al-Shehbaz et al. 2007).

Physoptychis Boiss. is one of the genera of *Alysseae* tribe, which comprises in all 24 genera with about 277 species (Španiel et al. 2015) distributed mainly in Eurasia (Rešetnik et al. 2013). Morphologically, the tribe *Alysseae* is characterized by a herbaceous or sub-shrubby growth form, a dense pubescence consisting usually of stellate trichomes, yellow or white (rarely pale pink) corollas, filaments usually possessing wings, teeth or other appendages, latiseptate or terete, mostly few-seeded siliques, often winged seeds and a base chromosome number of x=8 (Warwick et al. 2008, Rešetnik et al. 2013). In addition, a common physiological characteristic of the taxa of the tribe *Alysseae* is their ability to hyperaccumulate some heavy metals (Terenteva et al. 2014).

According to Davis (1965) and Cullen (1965), *Physoptychis* is morphologically related to the genera *Alyssoides* Adans. and *Fibigia* Medik. and it can be separated from *Fibigia* by having inflated fruits and narrower seed wings. On the other hand, possessing fruits in diameter ca. 20 mm, the genus *Physoptychis* is different from the genus *Alyssoides,* which has the fruits ca. 10 mm in diameter (Davis 1965). Recently, some phylogenetic investigations were carried out using molecular methods based on nuclear ITS regions (Warwick et al. 2008, 2010, German et al. 2009, Cecchi et al. 2010, Cecchi 2011) or nuclear and chloroplast DNA sequence data on the tribe *Alysseae* (Rešetnik et al. 2013). According to Rešetnik et al. (2013), *Physoptychis* is phylogenetically related to the genera *Alyssoides*, *Clastopus* Fairmaire, *Fibigia* and *Degenia* Hayek.

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The genus *Physoptychis* is represented by two species, *P. haussknechtii* Bornm. and *P. gnaphalodes* (DC.) Boiss. in Turkey (Davis 1965). While *P. haussknechtii* is an endemic species to Turkey known from only two localities in Sivas province, *P. gnaphalodes* is non-endemic, grows in the Caucasus, Iran, Northern Iraq and Turkey (Boissier 1867, Cullen 1965, Appel and Al-Shehbaz 2003). Recently, some other localities of *P. haussknechtii* were found in Sivas and Erzincan provinces. According to the Flora of Turkey (Davis 1965), *P. gnaphalodes* is known from one locality, in Hakkari province of Turkey, and grows in Armenia, Iraq and Iran. Botschantzeva (1976) treated *P. gnaphalodes* as synonym of *P. caspica* (Hablitz) V.V. Botscantz. In 2007, a new species for the genus, *P. purpurascens* Çelik & Akpulat very similar to *P. haussknechtii* was found and published. It has pink-purple petals, unlike the yellow petals known in *P. haussknechtii* from Sivas province of Turkey (Çelik et al. 2007). After this study, German et al. (2016) published a revision of the genus *Physoptychis* and found that Bornmüller's mention of yellow petals in *P. haussknechtii* was erroneous. Thus, *P. purpurascens* was reduced to being a synonym of *P. haussknechtii*. Currently, the genus *Physoptychis* is represented by two species (*P. haussknechtii* and *P. caspica*) in the world.

According to IUCN criteria *P. haussknechtii* is treated as an Endangered (EN) species in the Red data book of Turkish plants (Ekim et al. 2000). There is great deficiency of morphological knowledge on *P. haussknechtii* in the Flora of Turkey (Cullen 1965). The present study is the first comprehensive study of the morphology, and the first study of the anatomy, palynology, seed micromorphology and karyology of *P. haussknechtii*. Furthermore, the present study is the first comprehensive structural study not only of *P. haussknechtii*, but also of the genus *Physoptychis*.

There are only a few cytogenetic studies that have been carried out on *P. caspica* (syn. *P. gnaphalodes*) and the somatic chromosome number of this species is 2n=16 (Küpfer 1980, Maassoumi 1980, Carrique and Martínez 1984, Warwick and Al-Shehbaz 2006). There is no report on the karyology or ideogram of this species, so our study, conducted to determine the karyology and ideogram of *P. haussknechtii,* is the first study for the genus.

The aim of this study is to investigate the morphological, anatomical, palynological, micromorphological and karyological characteristics of the endangered Turkish endemic *P. haussknechtii*, and to reveal their contribution to the taxonomy of the genus.

Materials and methods

P. haussknechtii samples were collected during the flowering and fruiting period from different natural habitats in Sivas province. Localities of *P. haussknechtii* samples are listed in Tab. 1. Specimens for morphological studies were dried according to standard herbarium techniques and were stored in the Cumhuriyet University, Science Faculty Herbarium (CUFH). Identification was made according to Flora of Turkey (Davis et al. 1965) by the first author. Fresh samples were used for morphological measurements. The materials necessary for anatomical studies were collected from natural habitats and preserved in 70% alcohol. For anatomical analysis, transverse sections of root, stem, basal and cauline leaves were used. From these organs, hand sections were taken with a razor blade and sections were stained with Alcian blue (Sigma) for pectic substances and Safranine O (Sigma) for lignin substances in the ratio of 3:2. The sections were put in prepared dye about 5 minutes for staining. The well-stained sections were mounted in glycerin-gelatin to obtain permanent preparations (Jensen 1962). Sections were examined and measured by using an Olympus light microscope BX21. Microphotographs were captured using a digital camera Olympus DP70 connected with the Olympus BX51light microscope. For palynological studies, pollen grains were prepared for light microscopy (LM) by using the Wodehouse method (Wodehouse 1959). In micromorphological studies using scanning electron microscopy (SEM), the pollen grains, a part of the dried and scraped basal leaf, a part of the dried cauline leaf and mature seed were transferred to stubs prepared with double-sided adhesive tape. After that, the stubs were coated with the gold for 5 minutes. The surface ornamentation detail and the aperture characteristics of the pollen grains and surface characteristics of the leaves and seeds were investigated under the LEO 440 scanning electron microscope, and the micrographs were taken at different magnifi-

Tab. 1. Collection data of the investigated specimens of *Physoptychis haussknechtii.*

Locality	Collector and collector number	Date	Herbarium
B6 Sivas: Ulaş, Kurtlukaya village to Deliilyas village, 1474 m, 39°23’02.8˝N, 36°55’48.9″E	M. Tekin, 1244	12.06.2012	CUFH
	M. Tekin, 1408	11.05.2013	
	M. Tekin, 1565	05.06.2014	
	M. Tekin, 1542	16.05.2014	
	M. Tekin, 1654	30.04.2015	
B6 Sivas: Hafik, Gürlevik mountain, serpentine slopes, 1932 m, 39°34’31.8″N, 37°30’14.9″E	M. Tekin, 1591	11.06.2014	CUFH
	M. Tekin, 1662	11.05.2015	
B6 Sivas: Divriği-Kangal road, near Pınargözü village, serpentine slopes, 1549 m, 39°15’06.6″N, 37°41’06.1″E	M. Tekin, 1669	22.05.2015	CUFH
B7 Sivas: Divriği, Dumlucadağ mountain (Type locality), fer mine road, serpentine slopes, 1862 m, 39°23’32.0″N, 38°02’35.0″E	M. Tekin, 1670	22.05.2015	CUFH

cations. For palynological study, the terminology of Punt et al. (1994) was followed. The statistical analyses of anatomical measurements were made by the SPSS package program (ver. 15). For karyological analyses, root meristems from germinating seeds collected in the wild were used. The root tips were pretreated with α-monobromonaphthalene at 4 °C for 16 h. The root tips were fixed with Carnoy for 24 h at 4 °C. Before staining, the material was hydrolyzed with 1 N HCl for 12 minutes at room temperature. The chromosomes were stained with 2% acetic orcein and mounted in 45% acetic acid. Permanent slides were made by using the standard liquid nitrogen method. Microphotographs were taken through an Olympus BX51 light microscope. Chromosomes were classified according to the nomenclature of Levan et al. (1964). The ideogram was drawn based on centromeric index, and was arranged in decreasing order of size.

Results

Morphological characteristics

Perennial herb, plant height 5–22 cm. Caespitose, taproot thick with a few secondary branches. Stem much branched, covered densely with whitish grey trichomes and with numerous lateral sterile rosettes at the densely foliose base. Basal leaves 18–42×3–6.5 mm in dia., sessile, narrowly spatulate with subacute to obtuse apex and covered in whitish grey flattened stalked five-armed trichomes. Cauline leaves 20–55×2–4 mm in dia. sessile, linear to oblanceolate with subacute to obtuse apex, usually narrower than basal leaves and covered with whitish grey flattened stalked five-armed trichomes. Racemes densely flowered, up to 6 cm and 9 cm long at the flowering and fruiting stage, respectively. Pedicels up to 8 mm and 12 mm at the flowering and fruiting stage, respectively, and covered with whitish grey flattened stalked five-armed trichomes. Sepals 4–8×1.8–2.4 mm in dia., linear-oblong, rarely elliptic, outer side green-purplish with densely whitish grey flattened stalked five-armed trichomes, inner side green and smooth, margin membranous, apex obtuse to subacute. Petals 9–13× 2–3 mm in dia., spatulate with emarginate apex, pink-purple colored. Stamens dorsifixed, tetradynamous, long filaments 3.5–4.4 mm, short filaments 2.5–3.3 mm. Anthers 1–1.5 mm, longitudinal dehiscence. Pistils 3–4 mm, covered with whitish grey trichomes. Ovaries obovate or elliptic, 1.7–2.5×0.8–1.3 mm in dia., styles slender and distinct, stigma capitate. Siliculae conical in outline, 10–26×9–16 mm in dia., acuminate at apex, straw-colored at maturity and covered densely with whitish grey flattened stalked five-armed trichomes. Seeds 3–6×2–4 mm in dia., obovate-suborbicular, compressed, and pale brown. Seed margins membranous, wing-like and seed surface ornamentation coarsely reticulate (Figs. 1, 2J–K and Tab. 2).

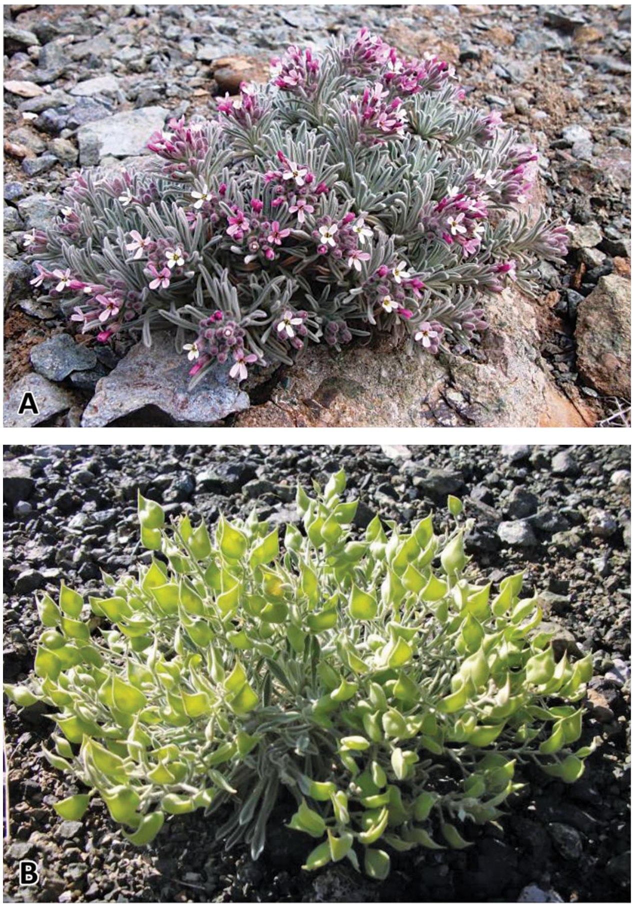

Fig. 1. General view of *Physoptychis haussknechtii* in flowering stage (A) and fruiting stage (B) in natural habitat (Photos by M. Tekin).

Palynological characteristics

The pollen grains of *P. haussknechtii* are radial symmetrical, isopolar and tricolpate. Pollen grain shape is elliptical in equatorial view (Figs. 2A, E) and orbicular in

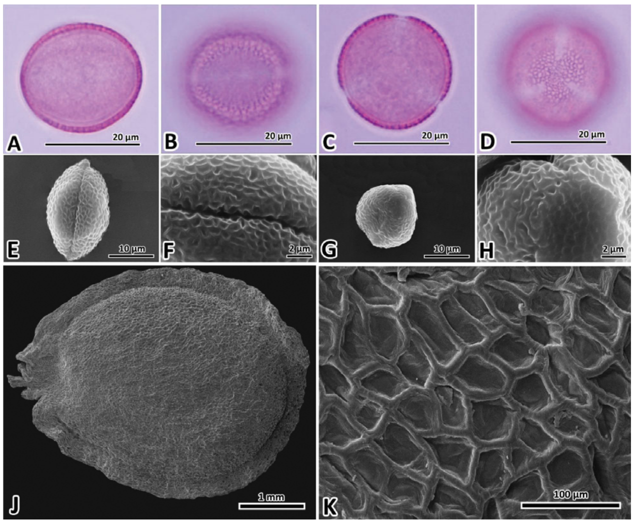

Fig. 2. Pollen grain microphotographs under light microscope (A–D) and scanning electron microscope (E–H), and seed micrographs under scanning electron microscope (J–K) of *Physoptychis haussknechtii*: A) equatorial view (high focus), B) aperture and exine ornamentation in equatorial view (low focus), C) polar view (high focus), D) apertures and exine ornamentation in polar view (low focus); E) equatorial view, F) aperture and exine ornamentation in equatorial area, G) polar view, H) apertures and exine ornamentation in polar area; J) general view of seed, K) ornamentation of seed coat.

Tab. 2. Morphological results comparison of *Physoptychis haussknechtii* and its synonym, *Physoptychis purpurascens*.

Characteristic	*P. haussknechtii* (Present study)	*P. purpurascens* (Çelik et al. 2007)
Plant height (cm)	5–22	15–18
Basal leaves dimension (mm)	18–42×3–6.5	25–40×3–5
Basal leaves shape	narrowly spatulate	narrowly spatulate to linear
Basal leaves apex	subacute to obtuse	obtuse to subacute
Cauline leaves dimension (mm)	20–55×2–4	–
Cauline leaves shape	linear to oblanceolate	lanceolate to linear
Cauline leaves apex	subacute to obtuse	–
Raceme length	up to 6 cm in flowering and 9 cm in fruiting time	up to 8 cm
Pedicel length	up to 8 mm in flowering and 12 mm in fruiting time	3–5 mm
Sepal dimension (mm)	4–8×1.8–2.4	3–5
Sepal shape	linear–oblong	linear–oblong
Petal dimensions (mm)	9–13×2–3	6–7
Petal shape	spatulate	obovate
Petal apex	emarginate	emarginate
Petal color	pink–purple	pink–purple
Long filament length (mm)	3.5–4.4	ca. 4
Short filament length (mm)	2.5–3.3	ca. 3
Anther length (mm)	1–1.5	2.5–3.5
Pistil length (mm)	3–4	–
Ovary dimension (mm)	1.7–2.5×0.8–1.3	–
Silicula dimension (mm)	10–26×9–16	20–25×10–12
Silicula shape	conical	conical
Seed dimension (mm)	3–6×2–4	3–4
Seed shape	obovate–suborbicular	suborbicular
Seed surface ornamentation	reticulate	smooth

polar view (Figs. 2C, G). The length of the polar axis (P) and equatorial diameter (E) are 24.11–29.34 µm and 21.02–25.38 µm, respectively. The ratio of P/E is 1.16 and pollen shape is subprolate. Exine thickness is 1.03–1.46 µm. Exine ornamentation is reticulate in both the equatorial and the polar sides (Figs. 2B, D, F, H).

Anatomical characteristics

Root: In the cross-section, the root exhibits a secondary structure. There is a periderm which makes up the outermost layer of the root and consists of multilayered dark colored cells, crushed, broken up and sometimes fallen out. Beneath the periderm, there is collenchyma tissue which consists of 2–5 cell layers, and cells are usually depressed elliptical, rarely rectangular or square shaped. Cortex is composed of 3–10 parenchymatous cell layers under the collenchyma. Cortex cells are usually elliptical, occasionally irregularly shaped and are bigger on the outer cross-section of the root than on the inner cross-section. Sometimes, there are wide gaps between the cortex cells. The phloem is located under the cortex, and is composed of 4–14 layered overlapping sequence of cells. Cambium cells are usually compressed, rectangular or irregular shaped, and are 2–4 layered between phloem and xylem. The region from the cambium layer to the root center consists of parenchymatic and tracheal cells belonging to xylem (Figs. 3A–C and Tab. 3).

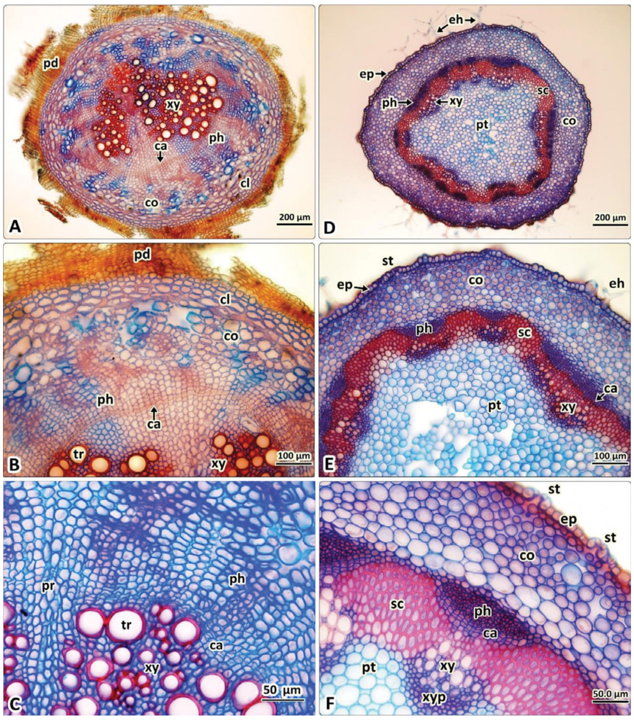

Fig. 3. Microphotographs of root (A–C) and stem (D–F) cross section of *Physoptychis haussknechtii*; ca – cambium, cl – collenchyma, co – cortex, eh – non-glandular trichome, ep – epidermis, pd – periderm, ph – phloem, pr – pith ray, pt – pith, sc – sclerenchyma, st – stoma, tr – trachea, xy – xylem, xyp – xylem parenchyma.

Tab. 3. The anatomical measurements of *Physoptychis haussknechtii*.

	Width (μm)		Length (μm)	
	Min–Max	Mean±SD	Min–Max	mean±SD
Root				
Collenchyma cells	7.95–32.33	17.78±6.96	12.86–74.60	32.34±18.46
Cortex cells	13.75–46.30	25.54±7.58	18.21–59.88	39.81±9.37
Cambium cells	2.59–8.96	4.79±1.58	8.12–18.92	14.38±2.53
Diameter of trachea	14.09–69.82	33.93±17.78	–	–
Stem				
Cuticle	0.31–0.87	0.53±0.18	–	–
Epidermis cells	4.65–18.24	11.95±3.85	11.50–23.41	17.25±3.30
Cortex cells	6.58–25.02	15.27±4.78	8.41–33.99	18.38±6.64
Phloem elements	1.60–5.86	3.21±1.11	2.04–8.31	4.29±1.54
Cambium cells	1.42–5.01	3.40±0.86	5.04–8.08	6.40±0.84
Pith cells	8.22–37.12	21.05±7.37	9.24–40.84	23.67±7.96
Diameter of trachea	10.58–25.47	15.30±3.88	–	–
Basal leaf				
Upper epidermis cells	10.69–26.81	19.00±3.81	11.25–43.82	25.09±8.04
Lower epidermis cells	9.33–22.99	15.37±3.92	11.23–26.85	17.83±4.36
Upper palisade parenchyma cells	14.85–32.21	21.61±4.07	26.54–53.70	39.59±6.24
Lower palisade parenchyma cells	12.14–28.58	20.17±3.91	26.63–76.16	45.83±11.00
Spongy parenchyma cells	18.68–31.80	25.48±3.50	21.95–45.03	33.17±5.62
Mesophyll tissue	231.96–329.66	278.94±32.58	–	–
Leaf	272.05–361.65	316.97±33.90	–	–
Cauline leaf				
Upper epidermis cells	10.12–31.90	20.38±5.21	16.15–42.29	24.67±6.12
Lower epidermis cells	8.91–21.77	15.60±3.59	11.39–33.06	19.48±4.28
Upper palisade parenchyma cells	20.34–46.81	29.18±5.43	35.23–82.44	48.65±9.68
Lower palisade parenchyma cells	12.72–37.18	21.10±5.79	24.94–61.88	40.89±9.11
Spongy parenchyma cells	14.56–43.38	27.52±6.70	16.61–53.20	34.09±8.24
Mesophyll tissue	324.34–381.06	348.41±12.75	–	–
Leaf	361.14–417.31	386.36±12.75	–	–

Stem: In the cross section of the middle part, the stem is ovoid shaped in outline and shows the primary structure. There is a thin cuticle layer on the uniseriate epidermis. The epidermis is composed of compactly arranged square, rectangular, elliptical or circular cells. Epidermis has stomata. Whitish grey trichomes are located intensely on the epidermis. Below epidermis, there is cortex tissue which consists of 7–18 layered elliptical or circular shaped parenchymatous cells. Outermost layer of cortex is composed of small collenchymatous cells (Fig. 3F). Under the cortex, there are phloem elements and sclerenchymatous tissue which forms continuous ring in the stem. The cambium is clearly distinguishable and is located between phloem and xylem elements. The cells of cambium tissue are typically rectangular shaped and are arranged as 2–5 layers. Under the cambium, xylem tissue occurs. The pith of the stem is formed from elliptical or circular parenchymatous cells. In some parts of the pith region, there are gaps which are formed by the fragmentation of the some pith parenchymatous cells (Figs. 3D–F and Tab. 3).

Basal leaf: There is a single-layered epidermis on the upper and lower surfaces of the leaf. There are unicellular flattened stalked five-armed trichomes and thin cuticle on both surfaces of the epidermises. Epidermis cells of both epidermis layers are oval or occasionally rectangular and square shaped. According to the mesophyll layer, leaf is equifacial. Upper palisade parenchyma consists of 2–4 cell layers, lower palisade parenchyma consists of 2–3 cell layers, and the shape of palisade parenchyma cells is cylindrical, rectangular or occasionally oval. Between the two palisade parenchyma layers, there is spongy parenchyma which consists of 2–5 cell layers. Spongy parenchyma cells are usually oval or almost circular shaped. Midrib is ovoid shaped, and is located close to the upper epidermis. Type of vascular bundle is collateral. All vascular bundles are surrounded by parenchymatous bundle sheath cells. Leaf is amphistomatic and stomata are anisocytic (Figs. 4A–B and Tab. 3).

Cauline leaf: In the cross section of the cauline leaf, there is single layered epidermis covered by unicellular flattened stalked five-armed trichomes and a thin cuticle layer

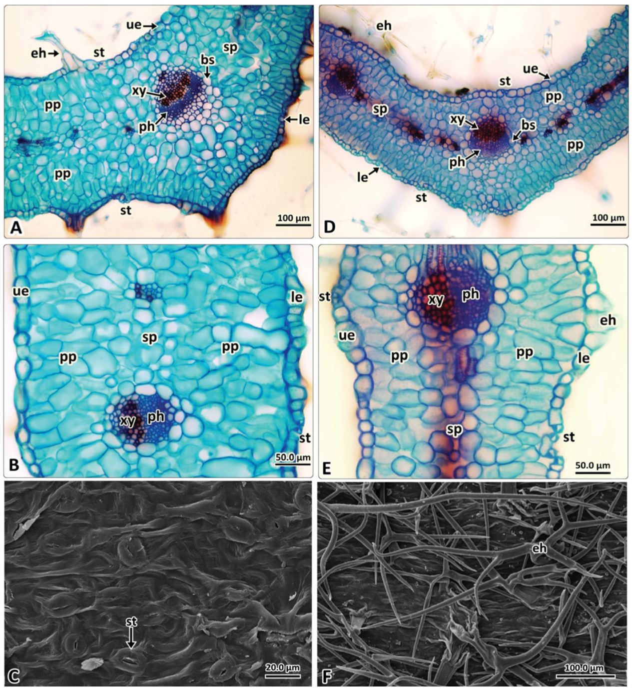

Fig. 4. Microphotographs of the leaf cross section under light microscopy (A, B, D, E) and micrographs of the leaf surface under scanning electron microscopy (C, F) of *Physoptychis haussknechtii*: (A–B) Cross section of the basal leaf, (C) View of basal leaf scraped upper surface, (D–E) Cross section of stem leaf, (F) View of stem leaf unscraped lower surface; bs – bundle sheath, eh – epidermal hair, le – lower epidermis, ph – phloem, pp – palisade parenchyma, sp – spongy parenchyma, st – stoma, ue – upper epidermis, xy – xylem.

on the upper and lower surfaces. Upper and lower epidermis cells are oval, rectangular or squarish. According to the mesophyll layer, the leaf is equifacial. Upper palisade parenchyma consists of 2–4 layered rectangular shaped cells, while the lower palisade parenchyma is composed of 2–3 layered cylindrical and rectangular shaped cells. Between the two palisade parenchyma layers, there is spongy parenchyma that consists of 2–4 layered oval or circular shaped cells. Midrib is triangular shaped. Type of vascular bundle is collateral and vascular bundles are surrounded by parenchymatous bundle sheath cells. Leaf is amphistomatic and stomata are anisocytic (Figs. 4D–E and Tab. 3).

Karyological characteristics

Our study has shown that the chromosome number of *P. haussknechtii* is 2n=16 (Fig. 5A). The shortest chromosome length is 2.14 µm, the longest is 6.01 µm, and haploid chromosome length is 31.90 µm. The chromosome arm ratios are measured as 1.15–2.58. The centromeric index varies between 2.74 and 7.81, relative lengths vary from 6.71 to 18.83. The karyotype formula of *P. haussknechtii* consists of five metacentric chromosome pairs and three submetacentric chromosome pairs. The ideogram is shown in Fig. 5B.

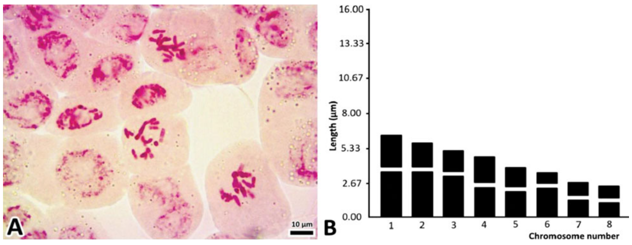

Fig. 5. The microphotograph of somatic chromosomes (A), and ideogram (B) of *Physoptychis haussknechtii*.

Discussion

Physoptychis is one of the small genus of the Brassicaceae family, and is described with two species, one of which, *P. haussknechtii,* is a Turkish endemic. Morphological knowledge on the genus *Physoptychis* in the Flora of Turkey (Cullen 1965) is deficient. The type species of the *Physoptychis* genus is *P. caspica*, a species that has yellow petals, so this characteristic has been used to specify the genus *Physoptychis* (Boissier 1867). According to German et al. (2016), a servant of Bornmüller collected plant samples from the mountain Dumlucadağ, in the Divriği district of Sivas province, Turkey in 1893, and Bornmüller published it as a new species with the name *P. haussknechtii* (Bornmüller 1899). Type samples of *P. haussknechtii* were all at the fruiting stage so *P. haussknechtii* was described solely on fruiting material. Because of its morphological similarity to the yellow flowered *P. caspica*, Bornmüller (1899) suggested this color was characteristic of the whole genus (German et al. 2016). As a result of German et al. (2016), this suggestion was found to be erroneous and the petal color of *P. haussknechtii* was determined to be pink-purple.

Morphological data on *P. haussknechtii* have been poorly described in Flora of Turkey (Cullen 1965), providing only information on general appearance of the species without measuring quantitative morphological characters. In the present study, all quantitative measurements such as height of stem, raceme, pedicel, long stamen, short stamen, anther, pistil and dimension of basal leaf, cauline leaf, sepal, petal, ovary, siliculae and seed have been carried out. In addition, in the study of Cullen (1965) the plant leaves were not distinguished as basal and cauline and all the leaves were described as being in shape narrowly spatulate to linear, but here, the leaves have been studied separately. In the present study, the shape of basal leaves is described as narrowly spatulate, but cauline leaves have been found to be narrower than basal leaves, linear to oblanceolate, unlike in the study of Cullen (1965). Only a few morphological characteristics such as the dense inflorescence and the conical siliculae with acuminate apices are results shared with the study of Cullen (1965).

Recent morphological studies on *P. haussknechtii* were made by Çelik et al. (2007) who described a new species *P. purpurascens* which was later reviewed to be a synonym of *P. haussknechtii*. They studied the specimens of only one population. In the present study, morphological characteristics of *P. haussknechtii* were studied from the specimens growing in four separate localities, one of which is the type locality, thus the distribution area of *P. haussknechtii* has been expanded (Tab. 1). When we compare our morphological results with the results of Çelik et al. (2007), there are some considerable differences, which are shown in Table 2. In addition, some morphological characteristics such as cauline leaf dimension, pistil length and ovary dimension of *P. haussknechtii* have been measured for the first time in the present study, and the morphological description of the species has been expanded.

Trichome morphology of eleven genera of tribe *Alysseae* was studied in Ančev and Goranova (2006). They recognized four trichome types: (1) simple, (2) stalked 2 to 5 armed, (3) stellate and (4) dendritic trichomes. In the present study, type (2) leaf trichomes were found on *P. haussknechtii*: they are 5 armed with a flattened stalk and smooth or minutely warty (Fig. 4F). According to the study of Ančev and Goranova (2006), 2–5 armed trichomes are present on the leaves of *Draba korabensis* Kümmerle & Degen, *Draba muralis* L., *Draba siliquosa* M. Bieb. and *Erophila verna* (L.) DC. subsp. *spathulata* (Láng) Walters which have leaf indumentum similar to *P. haussknechtii*, while stellate trichomes cover the leaves of some taxa belonging to the genera *Aurinia* Desv. and *Clypeola* L. and *Alyssoides* of the tribe *Alysseae*.

P. haussknechtii stem has the general anatomical characteristics of the Cruciferae family (Figs. 3D–F). They are in accordance with results of Metcalfe and Chalk (1950), who pointed out that the pith region is parenchymatous, and occupies a large proportion of the total diameter of the stem in the Cruciferae family. In addition, they stated that the stomata of Cruciferae family are of the cruciferous (anisocytic) type and those of *P. haussknechtii* are identical. As mentioned above, *Fibigia* is a genus related to *Physoptychis*, and morpho-anatomical adaptations of *Fibigia triquetra* (DC.) Boiss., which is endemic to Mid-Dalmatia area of Mediterranean region, were studied by Damjanović and Stevanović (1993). In a comparison of their results on *F. triquetra* and our results on *P. haussknechtii*, in the leaf anatomy of both species, mesophyll is equifacial, spongy parenchyma is weakly developed and stomata are tiny. While in the leaves of *F. triquetra*, upper and lower epidermis cells are equal in size and leaf surfaces are covered by stellate trichomes, in *P. haussknechtii* leaves, upper epidermis cells are usually larger than lower epidermis cells, and leaf surfaces are covered with flattened stalked 5 armed trichomes (Fig. 4F). The genus *Alyssum* L. belongs to the *Alysseae* tribe and it is related to *Physoptychis*. Anatomical characteristics of *Alyssum obtusifolium* Steven ex DC. were studied by Orcan and Binzet (2003). The results of our study show that *P. haussknechtii* has similar anatomical properties as *A. obtusifolium*, such as having roots in secondary structure, equifacial and amphistomatic leaves with anisocytic stomata. While the stem exhibits the primary structure and palisade parenchyma of the leaves have 2–4 layers in *P. haussknechtii*, the stem of *A. obtusifolium* exhibits the secondary structure and leaves have 2–3 cell layered palisade parenchyma. One of the Turkish endangered endemics is *Matthiola anchoniifolia* Hub.-Mor. which is a species of the *Anchonieae* tribe in Brassicaceae. The anatomy of *M. anchoniifolia* was studied by Tekin et al. (2013). While general root and stem anatomical characteristics of *P. haussknechtii* are similar to *M. anchoniifolia*, there are considerable differences in terms of leaf anatomy. The leaves of both species are equifacial, but palisade and spongy parenchyma in cauline leaves of *P. haussknechtii* consist of 2–4 cell layers, while the leaves of *M. anchoniifolia* have 1–2 cell layered palisade parenchyma, and 5–12 cell layered spongy parenchyma. These results have shown that the spongy parenchyma is weakly developed in *P. haussknechtii*, and well developed in *M. anchoniifolia*.

Brassicaceae is a stenopalynous family and polen grains are usually tricolpate and reticulate and pollen shape is varied more or less among genera within tribes but very rarely among species within the same genus (Erdtman 1972, Reile 1992, Abdel Khalik et al. 2002). The pollen grains of *P. haussknechtii* are tricolpate types, and exine ornamentation is reticulate in LM (Figs. 2A) and SEM (Figs. 2E), like the general pollen structure of the Brassicaceae family (Erdtman 1972). In addition, the pollen shape of *P. haussknechtii* was observed as subprolate, and pollen grains were radial symmetrical and isopolar. The genera *Degenia* and *Fibigia* are phylogenetically related to the *Physoptychis*. Pollen morphology of one of the Croatian critically endangered endemics *Degenia velebitica* (Degen) Hayek was studied by Mitić and Halbritter (2008). According to their study, *D. velebitica* has tricolpate pollen grains with reticulate exine ornamentation, and pollen shape is prolate. In present study, pollen grains of *P. haussknechtii* were found to be tricolpate with reticulate exine ornamentation, which is identical to *D. velebitica*. The pollen shape was found as a difference between the two species. While, pollen grains of *D. velebitica* are prolate, they are subprolate with 1.16 P/E ratio in *P. haussknechtii*. Palynological characteristics of some Turkish *Fibigia* taxa [*F. clypeata* (L.) Medik subsp. *clypeata* var. *clypeata*, *F. clypeata* subsp. *clypeata* var. *eriocarpa* (D.C.) Post, *F. clypeata* subsp. *anatolica* A. Duran & Tuştaş, *F. macrocarpa* (Boiss.) Boiss., *F. suffruticosa* (Vent.) Sweet] were studied by Çetin et al. (2012) and they found all pollens have tricolpate aperture type and reticulate exine ornamentation. These findings are identical for *P. haussknechtii*. Furthermore seed surface ornamentation of mentioned *Fibigia* taxa were reported as reticulate (Çetin et al. 2012) which is the same ornamentation for the seeds of *P. haussknechtii* (Fig. 2K).

In micromorphological study under SEM, basal leaf, cauline leaf and seed surface properties of *P. haussknechtii* were determined. All leaf surfaces were covered by dense unicellular flattened stalked 5 armed trichomes and had anisocytic stomata (Figs. 4C, F). The seed margins were membranous and wing-like and seed surfaces were observed as coarsely reticulate (Fig. 2J–K).

There are some karyological studies on the *Physoptychis* genus. The chromosome number of *P. caspica* and its synonym *P. gnaphalodes* were determined as 2n=16 (Küpfer 1980; Maassoumi 1980; Carrique and Martínez 1984; Warwick and Al-Shehbaz 2006). *P. haussknechtii* is related to *P. caspica* and we showed that the somatic chromosome number is the same, 2n=16. The base chromosome number of tribe *Alysseae* is reported as x=8 (Warwick et al. 2008, Rešetnik et al. 2013) There is no report on karyotype study of *Physoptychis* species. As the genus *Physoptychis*, *Alys-*

soides is one of the tribe *Alysseae* genera and there are some cytological studies on *Alyssoides* species such as *A. cretica* (L.) Medik., *A. graeca* Javorka, *A. sinuate* Medik. and *A. utriculata* (L.) Medik. and the chromosome numbers of all these species are reported as 2n=16 (Chichiricco and Tammaro 1980, Montmollin 1986, Nikolov 1991, Baltisberger 2006). The results of our karyological study show that the chromosome number of *P. haussknechtii* is identical to that of the mentioned related *Alyssoides* species. Also, one of the other genera of the *Alysseae* tribe is *Clastopus* Bunge ex Boiss. and the chromosome numbers of two species of this genus, *C. erubescens* Hausskn. and *C. vestitus* (Desv.) Boiss., were counted as 2n=16 (Maassoumi 1980; Khosravi and Maassoumi 1998). *Degenia* is one of the other genera of *Alysseae* tribe and chromosome number of taxa of this genus is reported as 2n=16 (Warwick and Al-Shehbaz 2006).

In a study conducted by Španiel et al. (2015), the tribe *Alysseae* includes 24 genera and 277 taxa, and they reported that the chromosome numbers and polyploid levels of 171 of them are known. Of these, 95 (55.6 %) taxa are diploids, 43 (25.1 %) are polyploids, and 33 (19.3 %) involve both diploids and polyploids. The most common base chromosome number in the tribe is x=8 and less frequently it is x=7. The highest variation in base chromosome numbers (x=7, 8, 11, 15) is found in the genus *Hormathophylla* (Španiel et al. 2015). In the present study, base and diploid chromosome number of *P. haussknechtii* was found as x=8 and 2n=16, respectively. These results are consistent with the mentioned literature. Španiel et al. (2015) determined that there are deficient karyotype analyses on the taxa of tribe *Alysseae* and suggested carrying out karyotype analyses on the taxa of this tribe. So, the karyotype analysis on *P. haussknechtii* is a significant event to eliminate this deficiency for the tribe *Alysseae*. One of the other genera of *Alysseae* tribe is *Fibigia* and chromosome number and karyotype analysis of some taxa of this genus such as *F. clypeata* subsp. *clypeata* var. *clypeata*, *F. clypeata* subsp. *clypeata* var. *eriocarpa*, *F. macrocarpa* and *F. suffruticosa* were studied by Çetin et al. (2012). While the chromosome number of these *Fibigia* taxa are identical with *P. haussknechtii*, karyotype analyses are quite different and karyotype formula of *P. haussknechtii* is not congruous with any *Fibigia* taxa of the study Çetin et al. (2012). In addition, results of chromosome measurement obtained for *P. haussknechtii* were quite different.

As a conclusion, in this study, morphological, anatomical, palynological, micromorphological and karyological characteristics of *P. haussknechtii,* an endangered endemic species for Turkey, were reported in detail for the first time. At the same time, this paper is the first comprehensive study conducted on the morphology, anatomy, pollen morphology, leaf and seed micromorphology and karyology of *Physoptychis* genus. In addition, all our results revealed by this study will contribute to the taxonomy of *P. haussknechtii* and *Physoptychis* genus.

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