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TAXONOMICAL, MORPHOLOGICAL, PALYNOLOGICAL, ANATOMICAL AND ECOLOGICAL INVESTIGATIONS ON MONOTYPIC GENUS *PACHYPHRAGMA* FROM TURKEY

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Abstract

The target of this work was to examine the taxonomical, morphological, anatomical, palynological, and ecological features and geographical distribution of the monotypic genus *Pachypragma* (*P. macrophyllum*) growing in Turkey. The flowering and fruiting periods of the species were recorded. Sampling was performed at different areas during 2015 to 2016. In the morphological investigation, the description of the genus was updated as a result of comprehensive evaluations of many samples, and distinguishing characteristics of the species. Moreover, micrographs of the seed and pollen surfaces of the species were taken using scanning electron microscope. The seed surface ornamentation was reticulate or alveolate-reticulate. The pollen grains were mostly radially, isopolar, and prolate, with polar axes of 18.36 ± 0.27 µm and equatorial axes of 12.11 ± 0.18 µm, and their outlines were oval in the equatorial axes and elliptical in the polar axes. The pollen grains were mostly tricolpate. The anatomical structures of the stem, root, and leaf were also studied. The geographical dispersion of the taxon is along the borders of Turkey. The physical and chemical features of the soil with live taxa of the studied taxon were ecologically examined.

The species risk category was determined in accordance with International Union for Conservation of Nature criteria.

Key words: *Pachyphragma macrophyllum*, SEM, Anatomy, Ecology, Pollen, Monotypic genus, Brassicaceae.

Introduction

Brassicaceae is a significant plant family due to it being an economic plant species with model systems, such as *Arabidopsis* L., *Brassica* L., and *Thlaspi* L. (Al-Shehbaz *et al*., 2006; Couvreur *et al*., 2010; Filiz *et al*., 2014). Brassicaceae comprises of 338 genera and 3709 species worldwide (Al-Shehbaz *et al*., 2006). It is represented by 571 species, with 65 subspecies, 24 varieties, and 660 taxa belonging to 91 genera in Turkey (Al-Shehbaz *et al*., 2007; Karaismailoğlu, 2016 and 2017).

Pachyphragma Reichb. is one of the monotypic genera of Brassicaceae that is mainly distributed in Caucasus (Guner *et al*., 2012). *Pachyphragma macrophyllum* (Hoffm.) Bush is the only species is also distributed in Turkey. It was treated in *Thlaspi* sensu lato (s.l.) under a monotypic section *Pachyphragma* DC. (Prantl, 1891). However, subsequent systematic studies showed that the genus *Pachypragma* is separated from *Thlaspi* s.1. by its rhizomatous habit and fruit morphology and it was a different genus closely related to *Thlaspi* (Schulz,1936; Davis,1965; Davie & Akeroyd, 1983; Mummenhoff *et al.*, 2001).

In the comprehensive literature investigation, some studies were performed on the species *Pachyphragma*, such as cytology (Davie & Akeroyd,1983), and molecular data such as the internal transcribed spacer sequence (Mummenhoff *et al*., 2001) to explain the phylogenetic correlations. With that exception, there was no eventual expectation of encountering any other work directly correlated to the genus. Due to the scarcity of data on the genus, it must be investigated as multidisciplinary. In this work, taxonomical, macromorphological, micro-morphological, anatomical, palynological, and ecological characters, and the International Union for Conservation of Nature (IUCN) risk category of this taxon in Turkey was studied for the first time and the identity information of the genus was revealed.

Materials and Methods

Specimens belonging to the taxon were collected from different areas between 2015 and 2016; their vouchers are given in Table 1 and their locations are indicated in Fig. 1. The specimens were defined according to Flora of Turkey and stored in the ISTF or M.C. Karaismailoğlu collections.

Macromorphological examinations such as fruit and seed characters were studied using an Olympus ZS51 stereomicroscope and Kameram imaging software. In the micromorphological examinations, seeds and pollen grains were prepared for scanning electron microscopy by pasting to stubs and covering them with gold. The terminology used was mostly compatible with that of Stearn (1985) and Punt (1994).

For the anatomical investigations, cross-sections were taken using a fully automatic microtome (Thermo Shonda Met Finesse) from the root, stem, and leaf. Subsequently, they were placed through a variety of alcohol and xylene series and stained with haematoxylin in a dying apparatus (ASC 720 Medite) and mounted with Entellan to observe their anatomical structures (Karaismailoğlu, 2015a, 2015b, and 2016). Anatomical features were monitored using an Olympus CX21FS1 microscope and Kameram imaging software.

The soil samples taken from the locations in Table 1 were obtained from 5 locations closest to the spread areas. The physical analyses were made in accordance with Chapman & Pratt (1961) and Karaismailoğlu (2016). Moreover, chemical tests were made following the procedure of Shirdam *et al.*, (2008) with some modifications. First, the soil samples were homogenized, and a 0.5 g sample was measured. Next, they were inserted into DAP 60 Teflon vessels and 10 mL of nitric acid was added to each vessel. Later, they were placed in a Berghof MWS-4 microwave oven, filtered, and measured by comparison with standard solutions in ICP-OES.

Table 1. Locations of the studied *P. macrophyllum* samples and collection data.

No.	Location	Voucher No.
1.	Rize-İspir, Kalkandere, roadsides, inclined slopes, 38 m, 15.06.2015	Karaismailoğlu, 278
2.	Rize-Çayeli, roadsides, woods, 16 m, 28.03.2015	Karaismailoğlu, 119
3.	Rize, Pazar, roadsides, 15 m, 28.03.2015	Karaismailoğlu,120
4.	Artvin-Hopa, 2. km, roadsides, open areas, 121 m, 06.03.2015	Karaismailoğlu, 97
5.	Artvin, Ardanuç, Kutul plateau, meadow, open areas, 1514 m, 08.05.2015	Karaismailoğlu, 150
6.	Artvin-Şavşat, 25. km, roadsides, 550 m, 06.03.2015	Karaismailoğlu, 99
7.	Trabzon, Hamsiköy, roadsides, meadow, open areas, 1950 m, 14.06.2016	Karaismailoğlu, 277

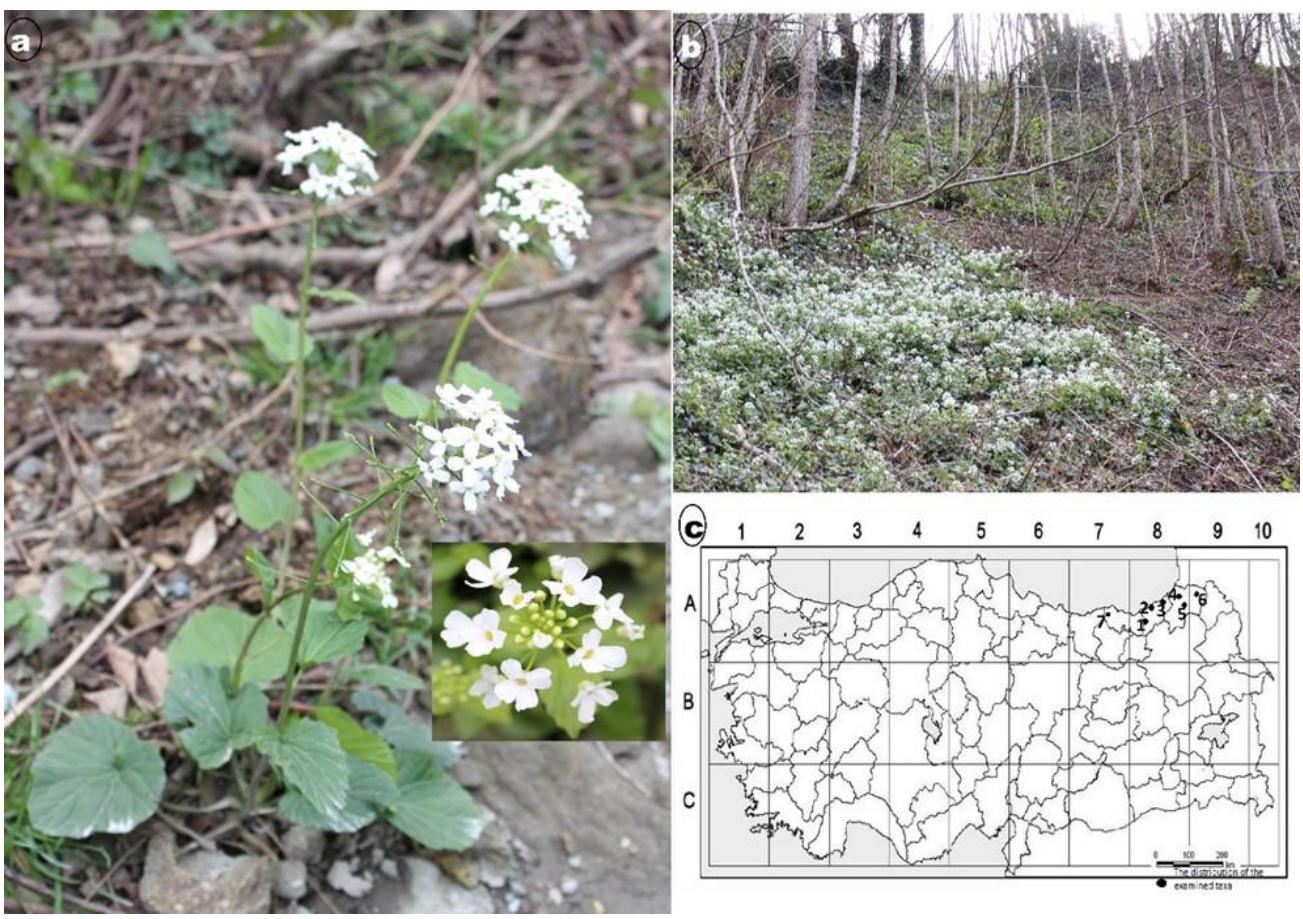

Fig. 1. *Pachyphragma macrophyllum*; a: Overview, b: Population view c: Distribution in Turkey.

Results and Discussion

The morphological features were updated by studying many characters from a large number of plants from the populations given in Table 1, in comparison with characteristics in Flora of Turkey (Davis, 1965). Accordingly, the description of the taxon examined is as follows;

Perennial, with creeping rhizome, woody or not, unbranched stems, 15–55 cm, glabrous, glaucescent. Basal leaves not rosette form, orbiculate-ovate, apex obtuse or rotund, petioles between 5–22 cm, leaf margins crenate, 1.5–6 cm (width) × 2–8 cm (length). Caulin leaves, orbiculate-ovate, not amplexicaul, shortly petiolate, between 0.5–3.5 cm, leaf margin crenate, apex obtuse or acute, size 1–3 × 1–4 cm (Fig. 1). Inflorescence raceme, elongating in fruit, racemes loose, 10–36 fruit, 5–14 cm in length. Sepals ovate-oblong, apex acute or obtuse, saccate, broadly membranous-margined green, 1–2× 2–3 mm. Petals white, spatulate, apex rotund, 3–5 veins, 3–5 × 6–9 mm. Stamens without appendages, anters yellow, 0.5–1 mm. Filaments narrow and linear, 1–2 mm, stigma capitate. Ovary ellipticate or ovate, 1–2 × 2–3 mm. Fruiting pedicels 5–22 mm, horizontally spreading. Fruits silicula. Siliculae very broadly obcordate, rotund at base, wing apex rotund, 8–14 × 6–18 mm. Wings broad, 0.5–2 mm. Apical sinus broadly shallow rounded, 0.2–0.4 mm. Style not exceeding sinus, 0.1–0.2 mm. Septum 3–5 × 6–9 mm. Seeds 2–4 in each loculus. Seeds ovate, light brown, 0.8–1.4 × 1.5–2.5 mm, rough surface, not mucilaginous (Fig. 2). Flowering time: March-May. Fruiting time: April–Jun. Distribution: Euxine forest, roadsides, open areas, and inclined slopes.

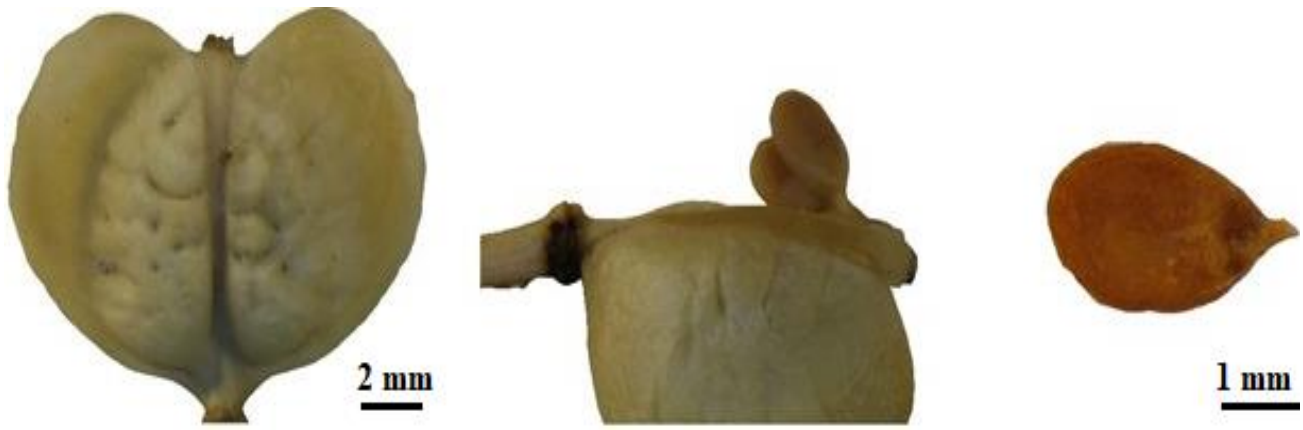

Fig. 2. Fruit, seed number per loculus, and seed of *Pachyphragma macrophyllum*.

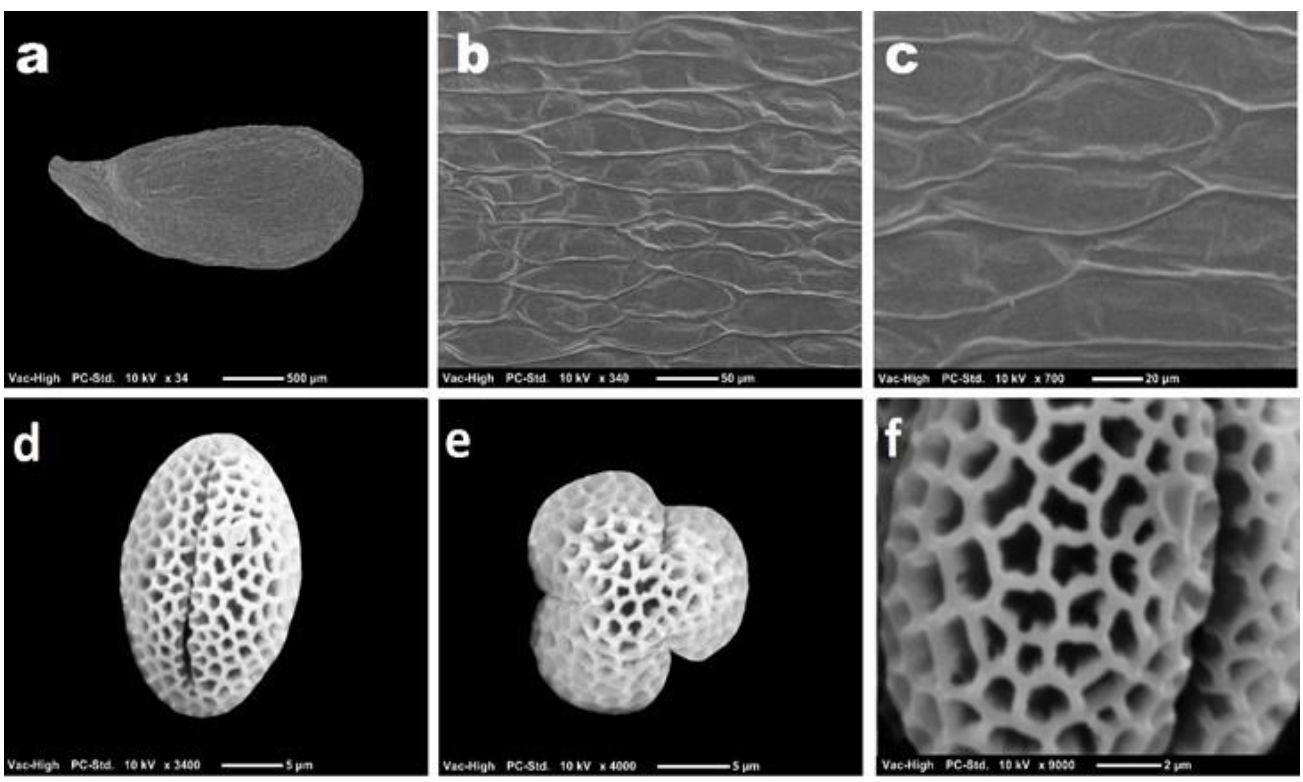

Fig. 3. Micromorphological structures of *Pachyphragma macrophyllum*; a: seed, b and c: seed surfaces, d: pollen (equatorial axes), e: pollen (polar axes), f: pollen surface.

Pachyphragma is a typical genus in terms of taxonomy, and intensive field work is needed to explain the status of this taxon. It appears that the description of this taxon was performed with very limited study in Flora of Turkey (Davis, 1965). New or additional information for some morphological characters, flowering-fruiting periods, distribution area, and habitat of the taxon were recorded in this study. Hence, the length, leaves, inflorescence, sepal, silicula, apical sinus and seed features of *P. macrophyllum* have been updated. The habitat, flowering, and fruiting time of the taxon are re-explained with some noteworthy differences.

The seed surface of the examined taxon was studied micromorphologically and surface structures, such as the surface ornamentations and cell types, and periclinal and anticlinal cell walls of the seeds, were analysed (Fig. 3). In this direction, the seed surface ornamentation type was determined as alveolate-reticulate (Fig. 3). Moreover, the cell shapes on the seed surfaces were diverse, and were alveolate or polygonal. The anticlinal cell walls in the studied taxon were sunken and flat or undulated, and the periclinal cell walls were convex or concave.

Micromorphological characters may present taxonomically valuable information (Heywood, 1971; Brochmann, 1992; Pinar *et al.*, 2009; Karaismailoğlu, 2015a and 2015b). Seed surface characters, including surface ornamentation, anticlinal and periclinal cell wall forms, and epidermal cell structure, have been found as supportive characters in the delimitation of taxa within some genera in the family Brassicaceae (Murley, 1951; Vaughan & Whitehouse, 1971; Barthlott, 1981, 1984; Koul *et al*., 2000; Moazzeni *et al*., 2007; Karaismailoğlu, 2016).

Table 2. Soil features of the examined samples.

Texture	1	2	3	4	5	6	7
	Clay-loam	Sandy-loam	Clay-loam	Clay-loam	Clay-loam	Sandy-loam	Clay-loam
Silt (%)	24.84	26.49	22.17	18.79	26.14	26.72	21.86
Clay (%)	43.49	33.64	46.26	41.15	40.89	30.65	38.91
Sand (%)	28.41	36.55	27.93	37.93	30.51	39.49	36.15
CaCO3 (%)	1.17	1.03	0.89	0.71	0.65	0.53	1.15
Organic matter	1.91	2.29	2.75	1.42	1.81	2.61	1.93
pH	4.54	3.84	5.65	6.80	6.47	5.11	3.62
Ca (ppm)	4.11	3.27	2.77	4.05	3.66	4.51	2.78
Mg (ppm)	5.76	5.14	4.43	6.08	3.77	7.15	4.96
Fe (ppm)	14.64	12.61	10.59	8.78	4.56	15.10	8.79
S (ppm)	8.73	6.42	5.77	6.42	9.14	8.65	4.11
P (ppm)	40.12	38.45	38.96	43.21	40.63	44.98	45.19
K (ppm)	3.76	4.99	6.70	8.54	8.05	9.12	6.53
B (ppm)	1.27	1.86	0.74	1.45	0.51	0.36	1.39
Zn (ppm)	6.54	5.76	3.86	1.24	8.29	6.54	7.55
Mn (ppm)	3.48	2.77	3.12	4.87	3.71	4.59	5.74
Cu (ppm)	3.11	2.86	1.88	1.55	2.64	3.44	3.21

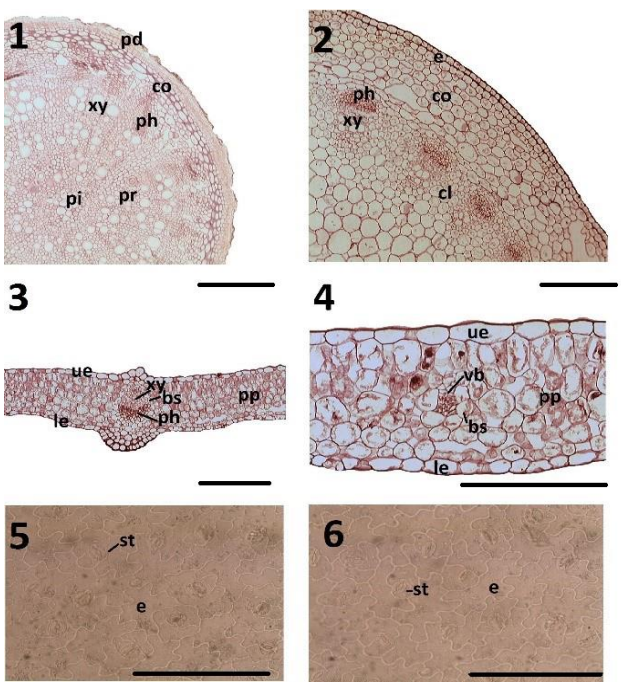

Fig. 4. Anatomical structures of *Pachyphragma macrophyllum*; 1: root, 2: stem, 3 and 4: leaf cross sections, leaf surface sections; 5: upper surface, 6: lower surface (pd: periderm, co: cortex, xy: xylem, ph: phloem, pi: pith region, pr: pith ray, cl: chlorenchyma, ue: upper epidermis, le: lower epidermis, pp: mesophyll parenchyma, bs: bundle sheet, vb: vascular bundle, st: stoma, scale bars: 100 µm).

In this work, the seed surface ornamentation was helpful as a good diagnostic characteristic at various taxonomical levels. The seed surface patterns of the examined taxon are reported for the first time herein. The surface ornamentation in this study was observed as reticulate or reticulate-alveolate, which is frequently found in some genera within the family Brassicaceae (Koul *et al*., 2000; Zeng *et al.*, 2004; Moazzeni *et al*., 2007; Karaismailoğlu, 2016). Additionally, it was also detected in some taxa of non-closely connected genera like *Erysimum* and *Camelina* (Murley, 1951).

The pollen morphological properties of the taxon were examined in detaile. The pollen grains are presented in Fig. 3. They were radially and isopolar and prolate in shape, with polar axes of 18.36 ± 0.27 µm and equatorial axes of 12.11 ± 0.18 µm, with oval outlines in the equatorial axes, and elliptical in the polar axes (amb) (Fig. 3). There were 3 (tricolpate) apertures in the studied pollen. Additionally, the colpus dimensions ranged from 13.15 to 15.21 µm in length, and from 0.75 to 1.21 µm in width. The margins were organized, and the ends were acute (Fig. 3). The exine thickness varied between 1.68 and 1.79 µm, and was mostly thicker in the apertural parts. Additionally, the intine thickness varied between 0.41 and 0.58 µm. The pollen ornamentation in *P. macrophyllum* was coarse reticulate with slightly meandering muri. The lumina consisted of polygonal or irregular cells and its diameter varied between 0.30 and 0.45 µm.

The pollen morphological feature data allowed us to better understand the practicality of pollen research in the separating closely related taxa. The grains of the *Pachyphragma* taxon could not be defined by any authors. The pollen shape in the examined taxa was prolate, which is the most widespread type in Brassicaceae, and is compatible with the results of Kalik *et al*., (2002), Mutlu & Erik (2012), and Karaismailoğlu (2017), who all examined the pollens of the various genera with in Brassicaceae.

The aperture and exine structure characteristics of the studied taxon were clarified as the main principle for the characterization of the phylogenetic relation in a lot of surveys in the literature (Kuprianova, 1967; Cronquist, 1968; Takhtajan, 1980).

The pollen grain exine ornamentation is very important for separating some closely-related taxa at the genus level in Brassicaceae (Khalik *et al*., 2002). In this study, the pollen ornamentation was coarse reticulate. According to Anchev & Deneva (1997), the exine ornamentations of the family Brassicaceae is mostly separated as reticulate and foveate. Their result is in line with the outcome in this study. Similarly, Mutlu & Erik (2012) reported that pollen grains, including coarse

reticulate (lumina of more than 1 µm), were found more in moist zones than in others, and a similar situation was detected in this work.

A multi-layered periderm was located on the outer surface of the *P. macrophyllum* root. Sclerenchyma bunches were located under the periderm. The cortex consisted of multilayer parenchymatic cells between 25 and 48 µm in diameter. The endodermis layer was not obvious. There was a visible cambium between the xylem and phloem. The most covered space in the roots was formed by secondary xylem. Pith rays extended from broad parenchymatic cells. In a cross-section taken from the stem, 2 epidermis layers were observed, consisting of flat or rectangular cells in outermost area. There was a 70- to 85-µm-thick cortex with 7–9 layers under the epidermis, consisting of ovoid-shaped cells. The Xylem and phloem elements were not very clear. The vascular bundle was open collateral. The vessel member diameter varied between 40 and 55 µm. In the inner layer, the essence consisted of large parenchymatic cells. In the abaxial and adaxial surfaces of the leaf, single-layer epidermis cells were observed, consisting of flat cells. The leaf was bifacial. The mesophyll layer was observed as having 3–5 spongy layers and a thickness of 60–70 µm, and 2–3 layered palisade parenchyma with a thickness of 25–40 µm. The leaves had collateral vascular bundles. The parenchymatic were cells surround by vascular bundles. On the leaf surfaces, the epidermal cells were irregular. The stomatal index was 33.33 in the upper surface and 30.15 in the lower surface. The stomata of *P. macrophyllum* are were anisocytic, and rarely anomocytic, which appeared to be at the same level as the epidermis. The stoma length differed within the studied specimens, varying between 8.15 and 11.14 µm.

Metcalfe & Chalk (1957) stated that anatomical properties and their usage is helpful in the taxonomy of the family Brassicaceae. Accordingly, anatomical characters of the root, stem, and leaf of *P. macrophyllum* were presented in this investigation for the first time (Fig. 4). The roots had a tinny periderm sheet in the outermost area, including large pith rays occurring with main xylem components and scleranchymatic structures. *P. macrophyllum* collenchyma cells were subsequent to a thick epidermis layer (14–25 µm) in the stem and resembled images of some *Alyssum*, *Erysimum*, and *Aubrieta* species (Orcan, 1997; Orcan & Binzet, 2003; Cansaran *et al*., 2007; Karaismailoğlu, 2016). The leaf was isobilateral. In the cross-sections of the leaf, the palisade parenchyma in the mesophyll layer included more space than spongy parenchyma, which was seen as decreased. The stomata in leaf was anisocytic, which also indicated Brassicaceae, as this extensively exists in this family (Cansaran *et al*., 2007). The anatomical characters of *P. macrophyllum* are important in creating the taxon's fingerprint, in the establishment of evolutionary relationships with closely related taxa, and in the questioning of the utilization of anatomical characters in taxonomical applications.

P. macrophyllum grows mostly in humid habitats at an altitude of 15–1950 m and it is distributed in forest regions. It is found together with *Euphorbia macroclada* Boiss. *Arabis caucasica* Willd. subsp. *caucasica*, *Arabis nova* Vill. *Barbarea vulgaris* R. Br., *Fagus orientalis* Lipsky, *Hesperis bicuspidate* (Willd.) Poiret, *Polygala major* Jacq., *Draba hispida* Willd., *Draba nemorosa* L., *Centaurea triumfettii* All., *Quercus pontica* C. Koch, *Artemisia annua* L., *Thlaspi huetii* Boiss., and *Thlaspi perfoliatum* L. Chemical and physical analyses of the soil specimens collected from locations in the native spreading fields of *P. macrophyllum* are presented in Table 2.

Physical investigations of the soil taken from 7 different populations of *P. Macrophyllum* indicated that this taxon typically choose slightly acidic or acidic, sandy-loam/clay-loam soils. Additionally, chemical investigations of the same soil samples showed that *P. macrophyllum* is not very selective in terms of organic substance. The soil is generally low in potassium (%), phosphorus (%), and calcium (%); however, it is more ordinary in typical conditions in terms of the other studied elements.

The examined taxon has been defined as least concern (LC) in accordance with the IUCN categories and criteria (2001), due to it being partly widespread and plentiful. This taxon is not threatened or in danger of extinction in accordance with IUCN categories.

In this work, the distribution of the monotypic genus *Pachyphragma* was determined in Turkey. A description of the examined genus with specimens including flowers, mature fruit, and seed was completed, and the new taxonomical characteristics of the genus were disclosed. Moreover, the micromorphological, anatomical, palynological, and ecological characters were examined and compared with closely related genera.

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