Micromorphological description of *Polygonum istanbulicum* (*Polygonaceae*)

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ABSTRACT
Polygonum istanbulicum, a species endemic to Istanbul was thoroughly examined in this study. Leaf epidermal characteristics, micromorphological structures belonging to stomata, pollen, and seeds were explained with the assistance of several SEM photographs, and the results obtained were compared to similar studies. Additionally, all specific and distinguishing characteristics of the species were also specified.

Keywords: *Polygonum istanbulicum*, stomata, pollen, seed, micromorphology.

Descripción micromorfológica de *Polygonum istanbulicum* (*Polygonaceae*)

RESUMEN
Polygonum istanbulicum, una especie endémica de Estambul, fue examinada a fondo en el presente estudio. Las características epidérmicas foliares, las estructuras micromorfológicas pertenecientes a los estomas, el polen y las semillas, se describieron con la ayuda de varias imágenes obtenidas mediante Microscopía Electrónica de Barrido, y los resultados obtenidos se compararon con estudios similares. Además, también se especificaron todas las características específicas y distintivas de la especie.

Palabras Claves: *Polygonum istanbulicum*, estoma, polen, semilla, micromorfología.

INTRODUCTION

The final list regarding the genus *Polygonum* s.l. was given by the first author in "Türkiye Bitkileri Listesi" [1]. Later, *P. melihae* was also published by Gemici and Tan [2], bringing the total number of species to 41.

Polygonum s.l. is a tremendously problematic genus for having their distinguishing characteristics being very close to each other. When inspected in the traditional sense, *Polygonum* used to include genera such as *Persicaria*, *Fallopia*, *Bistorta*, and *Koenigia* [3-5].

Polygonum istanbulicum M. Keskin is a local endemic species described from a single locality in the Anatolian side of Istanbul in 2009 by the first author [6]. It has an extremely narrow distribution, with constant and intense human pressure, and is under threat of extinction, placing it on the top of the list of species to be taken under immediate protection.

Micromorphology, despite being a feature that cannot be distinguished by the naked eye, offers vital information regarding the identification of species, such that, seeds showing similar characteristics under a light microscope can exhibit entirely different surficial treats when observed under Scanning Electron Microscopy (SEM). This study was carried out for the reason that, micromorphological data concerning *Polygonum istanbulicum* is not shared previously, adding to the current state of the species being extremely rare and prone to extinction.

MATERIAL AND METHODS

Samples constituting the study materials were obtained from the initial collection point (locus classicus) by the first author within the scope of his doctoral thesis on systematical, morphological, chronological, and sociological characteristics of the family *Polygonaceae*.

The collected samples are being preserved in MUFE herbarium.

Although the pollens were taken from a live plant sample, they were allowed to dry in room temperature prior to the coating process.

All studied parts of the plant material were coated with 16 nm thick Au-Pd mixture before being examined and photographed with Leo Morks EVO40 model Scanning Electron Microscope with magnifications varying between 65 and 10.000 magnification, 18 mm observation space, and a voltage between 10 and 20 kilovolts.

RESULTS AND DISCUSSION

Polygonum istanbulicum can be distinguished from other *Polygonum* species with its bushy structure, long stem, leaves with punctulate surfaces, large flowers, long pedicels, and achenes longer than the tepals [6].

The root system is highly sophisticated, entangled, and has a rigid woody structure. Roots don't penetrate deep into the soil and rather show horizontal growth. Rocks of volcanic origin are almost always present in the growing area of this species. The species has a distinctive mineral collection characteristic [7].

The stem can be as tall as 150 cm and demonstrates a rich embranchment from the base up.

Although the flowers are found along the entire stem, they are more concentrated on the upper parts. Flowers are white when they initially bloom (figure 1a), followed by a pink color (figure 1b), finally turning dark red after the pollination (figure 1c).

Pedicels have varying heights with the tallest ones towering the tepals (figure 1d).

Fig. 1. Whole Plant: a) General habit, b) Young pink flowers, c) Fruiting stage, d) Flowers with long pedicel.

The flowers exhibit eight stamens arranged in two whorls with filaments being broad at the base and narrow at the top (figure 2a).

Achenes are pyramidal-shaped and are significantly longer than tepals (figure 2b).

Fig. 2. Drawing of tepal: a) Dissected flower, b) Fruiting tepal. (Source: Keskin M. (2009) "*Polygonum istanbulicum* Keskin sp. nov. (Polygonaceae) from Turkey" *Nord. J. Bot.* 27(1):11-15)

Leaves.

Stomata are the basic structures of the leaf surface and the breathing apparatus of the leaves. Additionally, trichomes and other epidermal structures on leaf surfaces are important traits for identification.

The midrib is distinctive on the upper surface of the leaves in the *Polygonum* genus, whereas the veins are either slightly apparent or completely hidden. *P. istanbulicum* species fits in this description as well. The midrib can be clearly seen (figure 3a). Stomata are not present near the midrib, however, they start appearing along with the guard cells as the distance to midrib increases. Also, veins are usually present accompanying the midrib on the bottom surface of the leaf. Stomata are generally round-shaped with dimensions; 14.75-14.82 × 9.41-10.22 µ.

Guard cells are responsible for encompassing and guarding the stomata and are rather differentiated when compared with stomata. These guard cells are as numerous as epidermal cells because of the abundance of stomata.

Most *Polygonum* taxa generally prefer wetland habitats, but their habitats may shrink or dry out completely due to seasonal reasons. In such cases, the stomata regulates the vitally important water in a balanced manner.

When inspected carefully, the differentiation on the surface of the cells around the stomata is remarkable. There are numerous protrusions and asperities on the surface cells (figure 3b). However, the principal question regarding these structures is, whether they were existent when the plant was alive. These ornamentations may be a response of the plant to drying and coating since leaves are coated with heavy metals prior to SEM investigations.

Whatever the source of these ornamental structures is, it is obvious that SEM results will be utilizable as a stable trait in a systematic view as long as the same methods are used.

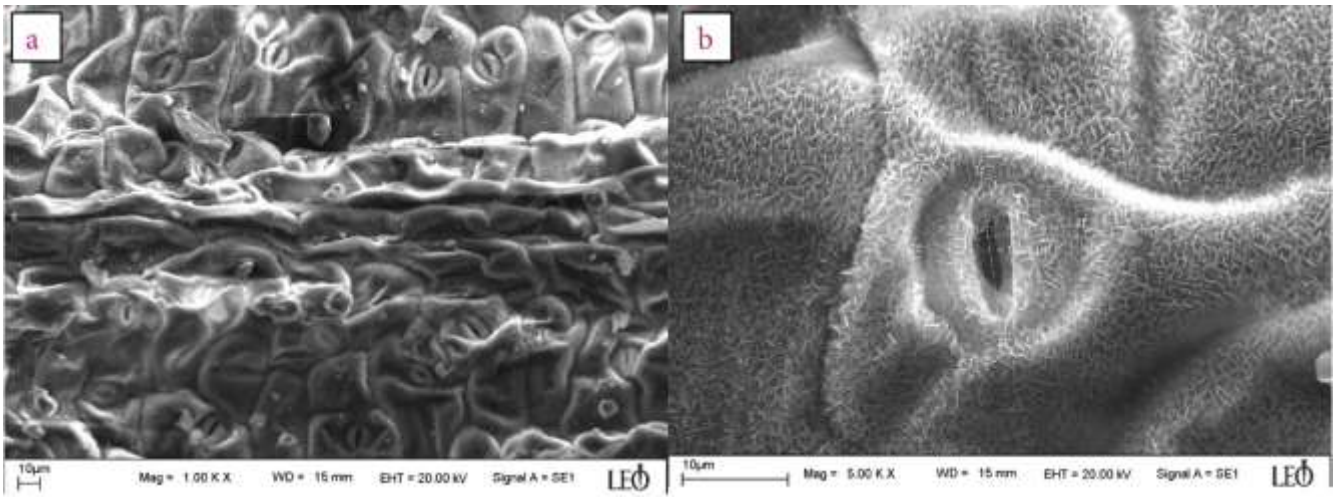

Fig. 3. Leaf: a) Surface, b) Single stomata.

Flowers.

It is possible to say that the floral structures of *Polygonum* species look very similar to each other. The pieces of perianth can't be named as sepals or petals since they are arranged in a single row and they do not show differentiation; hence they are called tepals. Free parts of the tepal lobes are generally longer than the tube itself.

Although the number of stamens is usually 8, different number of stamens can also be encountered within the genus. Stamens are usually oriented in two different rings. They are as tall as tepals at most. There is a single stylus and three short stigmas.

There are various studies on tepal micromorphology of the family *Polygonaceae*. These studies predominantly

investigated the epidermal and secretory cells on the surface of tepals [8-10].

Hong *et al.* [11] examined the tepal morphology of the family *Polygonaceae* systematically and revealed the relationship between its' genus and species.

P. istanbulicum also falls within the scope of these general rules. Flowers of this species have eight stamens arranged on two rings, three short stigmas, and a five-piece tepal with a tube shorter than the free parts of tepals (figure 2a).

A whole flower is displayed in figure 4a, where a filament with no anther can be seen between the tepals (2) and severed anthers around the flower are visible. Figure 4b, shows a photograph taken from inside the flower. Here 8 filaments (2) are arranged in two rings can be observed in addition to 4 anthers (1) that managed to stay on the filaments. Anthers are the largest in terms of length in the genus. Their lengths vary between 5.5 and 7.0 mm on average.

The tips of tepals are round cut, blunt, and slightly spoon-like.

Tepal surface became one of the most researched areas in the family *Polygonaceae* in recent years. In a study carried out by Shiha [12] surfaces of 15 species belonging to six genera were investigated, emphasizing the micromorphological treats in these species and also drawing attention to the differences between the species with numerous photographs and data.

The margins of surface cells in *P. istanbulicum* form a long, undulate, tightly joint and complex, puzzle-type surface system. The same situation was also reported by Shiha [12] in *P. equisetiforme* species, however *P. istanbulicum* is fairly differentiated with the length of the surface cells being longer than 10 times the width (figure 4d).

The inner surface of tepals includes numerous stomata (figure 4c) and these cannot be observed on the outer surface. When we take into consideration that tepals are leaf metamorphoses, it can be assumed that this high number of stomata (4) is rather normal.

Additionally, several pollen grains (3) fallen on the tepal surface can be seen in figure 4c.

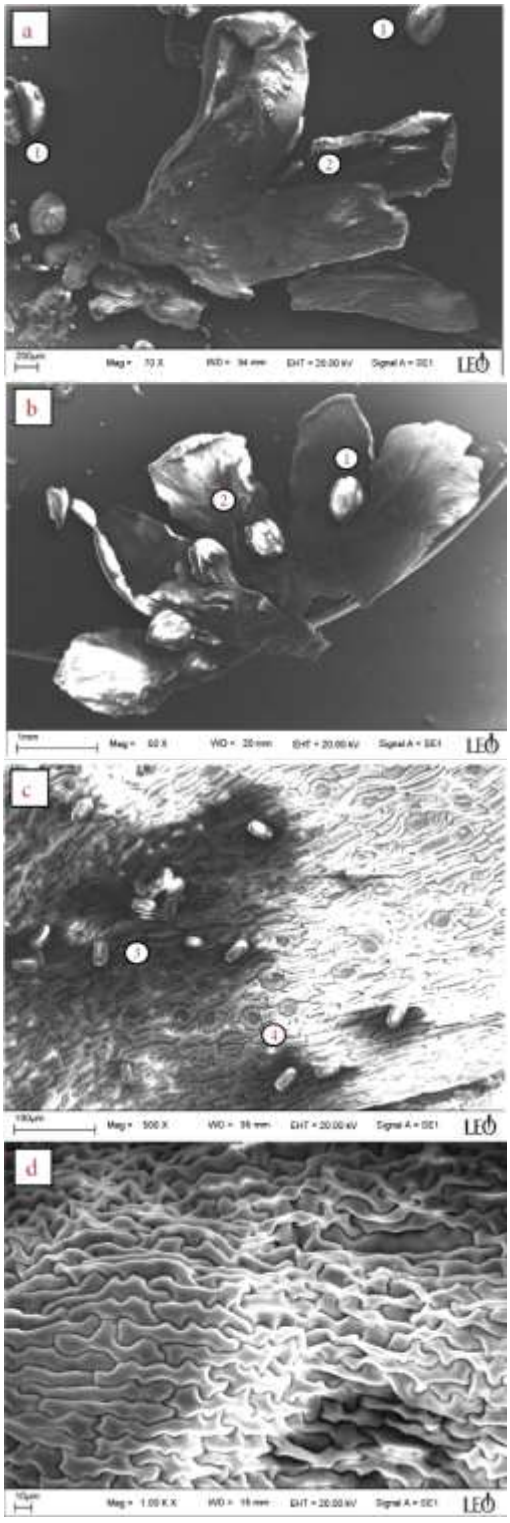

Fig. 4. Flower: a) Flower from outside view, 1. Anther, 2. Filament; b) Flower from inside view, 1. Anther, 2. Filament; c) Tepal inner surface: 3. Pollen, 4. Stomata; d) A detail of outer tepal surface.

Pollen.

Pollen is the final product of the reproductive organs of flowering plants. It is possible to say that there is a special pollen structure for every other plant. Pollens have been the focal point of several types of research throughout the years. There are numerous studies concerning the pollen characteristics of the genera of the family *Polygonaceae* carried out by different scientists [13-25]. Various classifications were made regarding the pollens. The general pollen type for *Polygonum* is *Avicularia* [14]. This classification was accepted in a comprehensive study carried out by Hong *et al.* [22]. Pollens of *Polygonum* are generally tricolporate or seldom hexa-colporate (especially *P. oxyspermum* subsp. *raii* = *P. mesembricum*) P=14.6 - 37.6 µ; E = 11.8 - 31.6 µ; mostly prolate and rarely prolate-spherical (P / E = 1.04 - 1.75). Pollens are mostly round or trigonal when looked upon from the poles [22].

It has been observed that these generalizations are also true for *P. istanbulicum.* Pollens are *Avicularia* type and present a tricolporate structure (figure 5).

The length of pollen grains is longer than the diameter. The measurements are 23.99-32.91 µ in length and 13.93-18.50 µ in diameter, average dimensions being 29.63 × 16.87 µ.

The width-length ratio was found as 1.756 [14] where it is stated that this ratio is applicable especially with *Avicularia* type pollens.

The surface of *P. istanbulicum* pollens, like other *Avicularia* type pollens, is coated with microspikes which has blunt tips (figure 5d). The dimensions of these spikes differ between 157.1 and 280.8 nm. Although not found very frequently, the distribution of these spikes is somewhat even. These spikes probably allow pollen to adhere more easily to the stigma.

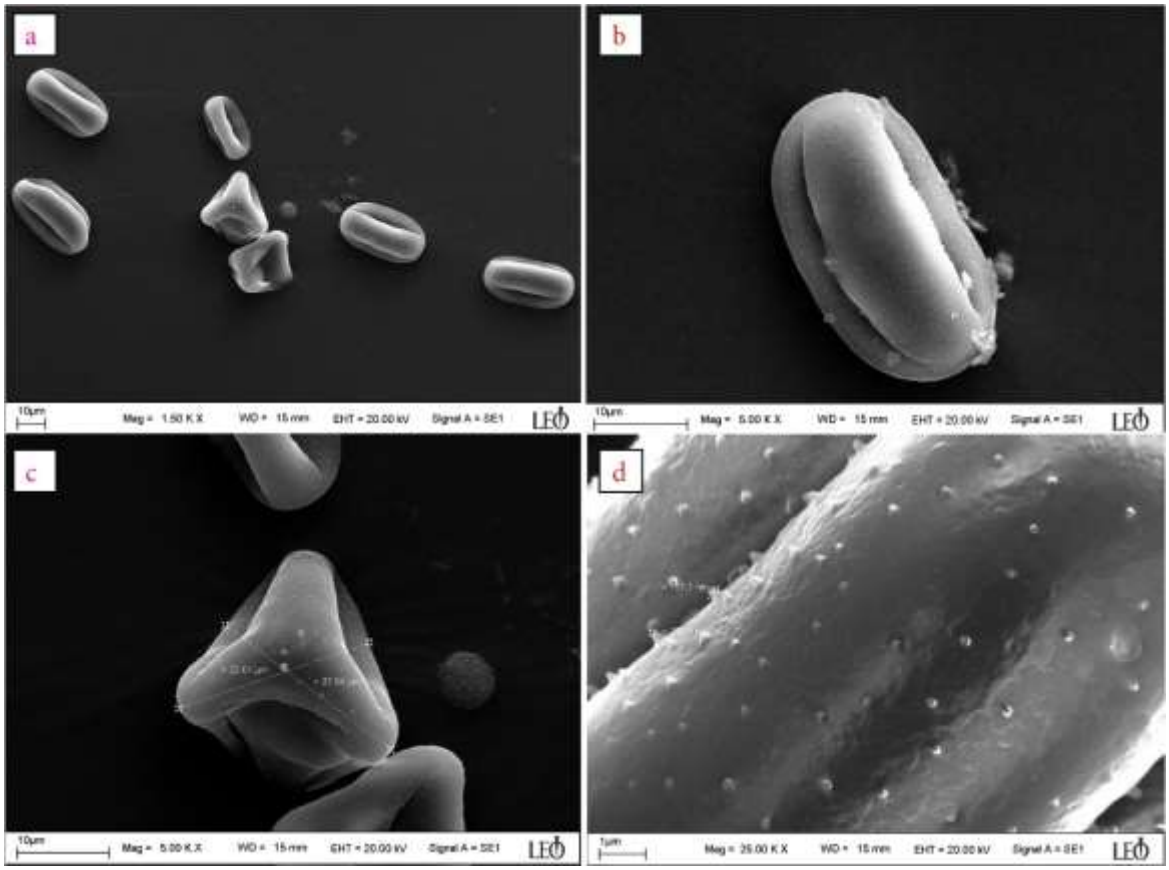

Fig. 5. Pollen: a) Several dry pollens, b) Lateral view of a single pollen, c) Polar view of a single pollen, d) Detailed view of pollen apertures.

Seed.

The seed is the final point of the reproduction of flowering plants, merely their baby, and the guarantee of their lineage. Seed occupies an important place in the genus *Polygonum* and is an indispensable characteristic in identification.

Especially in annual species, flowers produce normal types of seeds in typical seasons such as between spring and summer, whereas the seeds are a little distorted, slightly narrow, and longer at other times. As a result of this feature, the morphological differences in the seeds of plants especially collected in late summer, when compared to seeds of regular seasons, cause them to be named differently leading to the abundance of synonyms in annual species. Leaf characteristics are generally similar to seed characteristics.

When viewed from the outside the seed shows very little differentiation. Differences such as roughness or smoothness of the seed surface, whether it is glossy or matte can only be apparent when examined under SEM with high magnifications. For this reason, seeds have been the main topic of many scientific articles [26-36].

Polygonum s.str. features achenes that are unopenable and comprising three carpels. Achenes are surrounded by tepals either fully or partially at the maturity stage. The colour of mature achenes can be either glossy or matte, but these features have little taxonomical importance. Surface characteristics of achenes have been the subject of many studies [10, 27, 31, 35].

Seeds of *P. istanbulicum* (figure 6a) are 6 mm long trigonal – pyramidal shaped, brown, matte, and smooth, with a protruding tip and significantly longer than tepals.

The surface (figure 6b), which appears to be very smooth under the light microscope was observed to have wide and small pimple-like protrusions and blunt-tipped bumps and barrows when investigated under SEM.

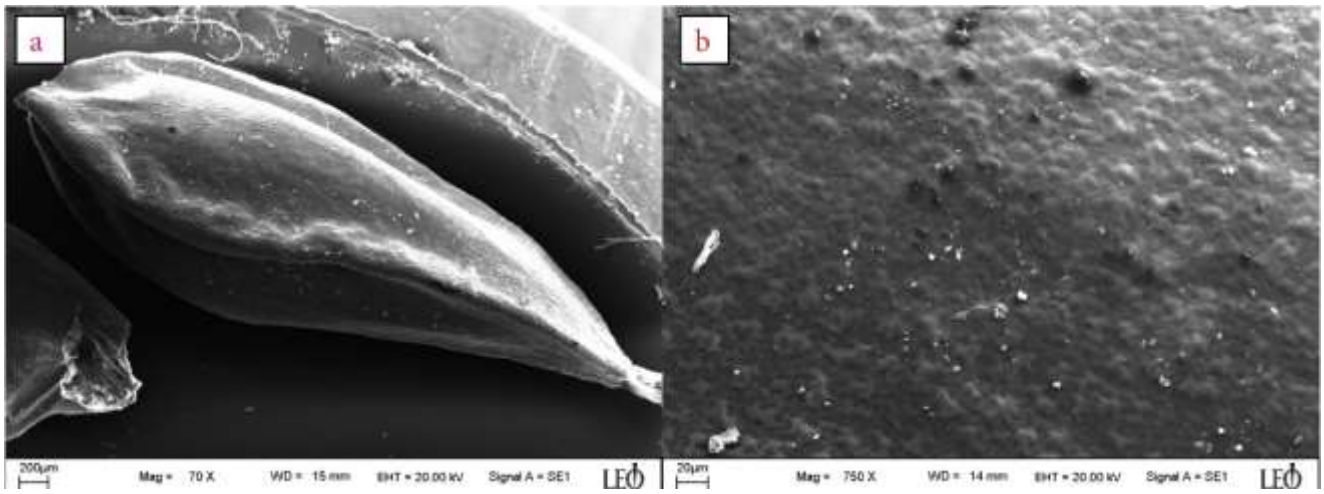

Fig. 6. Seed: a) General view, b) A detail of seed surface.

CONCLUSIONS

P. istanbulicum distinctive macromorphological structure already having specific attributes within the genus [6], was presented comprehensively in this study regarding its micromorphological attributes.

Pollens of *P. istanbulicum* species were of *Aviculare* type with the length/width ratio determined as 1.759. Pollen characteristics are rather congruent with the pollen features stated in other *Polygonum* studies [10, 22, 25, 37].

The seeds of *P. istanbulicum* are noticeably characteristic with a length of 6 mm and being longer than the tepals.

Tepal characteristics were found to have a considerable taxonomic importance [11]. Tepals of *P. istanbulicum* also have their characteristic features with a puzzle-like appearance.

Anatomical features of the Polygonaceae family were thoroughly investigated by Ayodele & Olowokudejo [33]. Significant epidermal differences between the studied species were reported in their research. The morphology and arrangement of epidermal cells showed a lot of dissimilarities. They developed a key using these differences that they observed in their studies.

In the light of this study and other similar studies mentioned in this article, it is considered that examination of the characteristics of at least closely related species of Turkish *Polygonum* s.str. in general, would be appropriate.

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