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Anatomical, micromorphological and karyological contributions to the *Periploca graeca* (*Apocynaceae*) taxon

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Periploca graeca (*Apocynaceae*) taksonuna ilişkin anatomik, mikromorfolojik ve karyolojik katkılar

Abstract: *Periploca graeca* L. (Garipler urganı) is currently treated as a species-level taxon without any infraspecific taxa in modern classifications. In this study, comprehensive stem and leaf anatomy and micromorphology, and somatic chromosome number of *P. graeca* taxa, collected from different populations in Türkiye, were examined comparatively, for the first time, using light microscopy and Scanning Electron Microscopy (SEM). In anatomical examinations, stems contained numerous groups of small, thick-walled cortical sclerenchymatic fibers; bifacial and hypostomatic leaves included anomocytic and parasitic stomata; and bicollateral vascular bundles, druse crystals, and laticifers were found throughout stems and leaves. In SEM examinations, stems were glabrous, petiole and lamina surfaces were covered with densely villous indumenta, or relatively glabrous, and epicuticular wax layer was rough on the stem and petiole, and radially striated on the leaf blade. Non-articulated laticifers, located throughout the midrib, petiole and stem, and "colleters", known as glandular hairs located at the base of the petiole (defined as the standard type), were determined as the two main glandular structures. The somatic chromosome number was determined as 2n = 22 for the both populations. Parametric and non-parametric analyses indicated that population-level variation in *P. graeca* was mainly associated with differences in tissue dimensions of stem (periderm, collenchyma, cortex and vascular bundles) and mesophyll (palisade and spongy), and size and density of epidermal cells and stomata. Although some characters showed statistically significant differences, the overall anatomical, micromorphological and karyological similarity between the populations suggests that these differences are related to environmental variation rather than taxonomic differentiation.

Key words: Anatomy, chromosome number, leaf, micromorphology, stem

Özet: *Periploca graeca* L. (Gariplerurganı), modern sınıflandırmalarda herhangi bir tür altı takson içermeyen, tür düzeyinde ele alınan bir takson olarak kabul edilmektedir. Bu araştırmada Türkiye'deki farklı popülasyonlardan toplanan *P. graeca* taksonlarının kapsamlı gövde ve yaprak anatomisi ve mikromorfolojisi ile somatik kromozom sayısı ışık mikroskobu ve Taramalı Elektron Mikroskobu (SEM) kullanarak ilk kez karşılaştırmalı olarak incelenmiştir. Anatomik incelemeler, gövdelerde korteks boyunca çok sayıda küçük kalın duvarlı sklerenkimatik lif gruplarının bulunduğunu, bifasiyal ve hipostomatik tipteki yaprakların anomositik ve parasitik stomalar içerdiğini, gövde ve yaprak boyunca bikollateral iletim demetleri, druz kristalleri ve latisiferlerin bulunduğunu göstermiştir. SEM incelemelerinde, gövdelerin tüysüz olduğu, yaprak sapı ve yaprak yüzeylerinin yoğun villoz tüy örtüsü ile kaplı olduğu, ya da nispeten tüysüz, epikutikular mum tabakasının gövde ye yaprak sapında pürüzlü yaprak ayasında ise radyal çizgili olduğu tespit edilmiştir. Ortadamar, yaprak sapı ve gövde boyunca yer alan eklemsiz latisiferler ile yaprak sapının tabanında konumlanan ve salgı tüyü olarak bilinen "colleterler" (standart tip olarak tanımlanan) iki önemli salgı yapısı olarak tespit edildi. İncelenen her iki populasyon için somatik kromozom sayısı 2n = 22 olarak belirlenmiştir. Parametrik ve parametrik olmayan analizler, *P. graeca*'daki popülasyon düzeyindeki varyasyonun esas olarak gövde (periderm, kollenkima, korteks ve iletim demetleri) ve mezofil (palisad ve sünger) dokularının boyutlarındaki ve epidermal hücrelerin ve stomaların boyut ve yoğunluğundaki farklılıklarla ilişkili olduğunu göstermiştir. Bazı karakterler istatistiksel olarak anlamlı farklılıklar gösterse de, popülasyonlar arasındaki genel anatomik, mikromorfolojik ve karyolojik benzerlik, bu farklılıkların taksonomik farklılaşmadan ziyade çevresel varyasyonla ilgili olduğunu işaret etmektedir.

Anahtar Kelimeler: Anatomi, kromozom sayısı, yaprak, mikromorfoloji, gövde

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1. Introduction

The subfamily Periplocoideae is a relatively small taxon group of the *Apocynaceae* with mostly woody perennials as lianas, shrubs or small trees, rarely erect, scrambling or twining herbs, or geophytes with underground tuber. Periplocoideae is represented by approximately 33 genera and 200 species in tropical and subtropical regions, including tropical Africa, Madagascar, Eurasia and Australia (Endress et al., 2019). *Periploca* L., the subject of this research, is one of the commonly distributed genera of the Periplocoideae. The most recent revision of the genus *Periploca* was made by Venter (1997), who described 14 natural species from Africa, Asia, and Europe, consisting of mostly climbing vines and occasionally erect shrubs growing in diverse habitats ranging from moist forests to drier savannas or xeric shrublands.

Periploca graeca L. is a deciduous, climbing shrub characterized with numerous branched, latex-bearing young shoots reaching the height of 30 m, opposite, elliptical, ovate to ovate-lanceolate leaves, inflorescences of 3–30-flowered cymose with 25-60 mm peduncles, rotate corollas composed of purple-violet petals with long, villous margins, a pair of follicles, acutely separated and slightly fused at the tip, and numerous, small, brown seeds with a silky coma at the tip. *P. graeca* which is known with the local name "Gariplerurganı" in Türkiye, has a wide distribution in our country especially on coastal areas but also spreads in humid areas of the interior parts (Güner, 2012; Güven and Sarıkaya, 2024).

The first detailed taxonomic treatment on Turkish Periplocoideae taxa was made by Browicz (1978), who recognised two Periplocoideae genera (*Periploca* and *Cyprinia* Browicz) under the distinct family *Asclepiadaceae*. Thereafter, Güner (2012) listed all of the *Asclepiadaceae* members under the *Apocynaceae* s.l. In the Flora of Türkiye, of the two Periplocoideae genera, *Periploca* included *P. graeca* L. var. *graeca* and *P. graeca* var. *vestita* Rohlena, which were distinguished by only leaf and petiole pubescence, and the other one, a monotypic genus *Cyprinia*, comprised only *C. gracilis* (Boiss.) Browicz (Browicz, 1978; Güner, 2012). As seen in the literature above, the previous intraspecific delimitation of the *P. graeca* taxa was based solely on the pubescence type and density of leaf and petiole (Browicz, 1978).

However, later taxonomic studies on a larger taxon group of the species revealed that pubescence characteristics, which are known to be environmentally plastic and developmentally variable, were not sufficient to differentiate the taxa at the variety level (Venter, 1997). Furthermore, detailed morphological (Güven and Sarıkaya, 2024) and fruit and seed micromorphological data (Sarıkaya and Güven, 2024) did not provide a clear distinction for the varieties in question. Accordingly, in the current classification, *P. graeca* is treated as a single, species, and *P. graeca* var. *vestita* is regarded as a synonym of *P. graeca* (POWO, 2026). Nevertheless, because the historical varietal delimitation was based exclusively on pubescence-related characters, which are known to exhibit considerable variation among populations growing under different ecological conditions, further anatomical, micromorphological and karyological investigations are required to test whether these characters and their associated traits reflect stable taxonomic differences or environmentally driven intraspecific variation.

Taxonomic studies on *Periploca* taxa, including *P. graeca*, showed that the shape, color, and pubescence of the corolla and corona, as well as the shape and size of the tetrads and pollinators, had frequently been used as key characters in distinguishing the members of the genus (Venter, 1997; Verhoeven and Venter, 1994, 1998; Heneidak and Naidoo, 2015). Pollen morphology of 13 *Periploca* taxa, including *P. graeca*, was examined using SEM, and the taxa were determined to be similar by means of tetrad arrangement (rhomboid, decussate, or linear), with minor differences in tetrad size (Verhoeven and Venter, 1994). Additionally, most of *Periploca* members which are known for their rich biochemical content, have been used in folk medicines since ancient times. Phytochemical researches on the *Periploca* taxa pointed out that the members of the genus exhibited various cardiotonic, anti-inflammatory, immunosuppressive, antitumor, antimicrobial, antioxidant, insecticidal and other properties (Huang et al., 2019). Nevertheless, it was stated that the plant contains a white, poisonous latex turning black when dry, and causes a burning sensation when in contact with the skin. In addition to its medicinal and toxic properties, *P. graeca* is known as an ornamental plant widely preferred as an ivy hedge in gardens (Demirci et al., 1998).

Recent advances in imaging technologies have enabled anatomical and micromorphological features to become widely preferred distinguishing characteristics for the identification and species-level delimitation of plant taxa. Some anatomical and micromorphological studies on the members of *Periplocoideae* as *Tacazzea* Decne. (Venter et al., 1990a) and *Petopentia* Bullock (Venter et al., 1990b), pointed out that these characters made significant contributions to resolving taxonomic problems among taxa of the subfamily. The wood anatomy of 56 species of *Apocynoideae* and eight species of *Periplocoideae,* including *P. graeca*, was investigated in phylogenetic aspect, and it was determined that the wood anatomy was rather homogeneous within the nonclimbing and climbing lineages (Lens et al., 2009). Although detailed wood anatomy of *P. graeca* was investigated by Lens et al. (2009), no comprehensive investigation presenting the variation in anatomical and micromorphological characteristics of the primary stem and leaf within the species has been available. A more recent study on *Periploca angustifolia* Labill, which was identified laticifer distribution in vegetative and reproductive organs, provided the general anatomical structure of the species (Dghim et al., 2015). Additionally, several recent micromorphological and anatomical studies on the members of Turkish *Apocynaceae* genera such as *Araujia* Brot. (Güven, 2026), *Cionura* Griseb. (Güven, 2025a) and *Vincetoxicum* Wolf (İlçim et al., 2010; Güven 2025b) have also significantly supported to anatomical and micromorphological knowledge of the *Apocynaceae*.

Chromosome number data is another systematic characteristic constantly used for systematic purposes for plant taxa. Previous chromosome count data related to *P. graeca* based on reports of many researchers, which were obtained from the online database Chromosome Counts Database / CCDB (2026), displayed the chromosome numbers of $2n = 22$ and $2n = 24$ for this species. This study aims to present the first somatic chromosome number data for *P. graeca* taxa from Türkiye.

To our knowledge, no comprehensive anatomical and micromorphological data, and chromosome number counts related to *P. graeca* have been conducted, although infraspecific delimitation within the species has been questioned in later taxonomic treatments by Venter (1997) and Güven and Sarıkaya (2024), and the characters previously used for this purpose have been considered variable. Furthermore, studying the anatomical and micromorphological structure of such medicinal and aromatic plants can contribute to the systematics of the taxa and provide important information regarding the source of phytochemical content and physiological characteristics of other members of the family (Manzoor et al., 2023). In addition, examination of the internal (laticifers) and external glandular structures (colleters) of such a poisonous plant in detail may provide new data about plant structures that are effective in toxicity. In the present study, individuals showing differences in leaf and petiole pubescence were intentionally selected from different populations and habitats, not with the aim of proposing infraspecific taxa, but to evaluate whether anatomical, micromorphological and karyological characters associated with this historically used trait exhibit consistent patterns.

This sampling strategy also allowed an assessment of the distribution of pubescent individuals beyond the regions previously reported in the Flora of Türkiye. The present study aims to count somatic chromosomes and to examine the stem and leaf anatomy and micromorphology of *P. graeca* individuals collected from different populations in Türkiye, in order to assess the extent of variation in these characters and to contribute additional anatomical and cytological data to the systematic understanding of this species.

2. Materials and Method

2.1. Plant materials

Periploca graeca specimens were collected from two distinct natural habitats in Türkiye: Kastamonu, Cide, Himmetbeşe Village, around of hazelnut orchard, 320 m, 18.10.2024, S. Güven 206, RUB!; Trabzon, Sürmene Çamburnu Neighborhood, around of Faculty of Marine Sciences, along roadside, 35 m, 25.10.2024, S. Güven 207, RUB!. The collected plant specimens were identified using the Flora of Turkey and the Eastern Aegean Islands (Browicz, 1978), prepared as herbarium specimens, and kept in the Herbarium of the Department of Biology of Recep Tayyip Erdoğan University (RUB). The images of the studied specimens in their natural fields are given in Figure 1, and the meteorological data related to the localities where they were collected are shown in Table 1.

2.2. Anatomical protocols

For anatomical and micromorphological studies, 15 mature stem and leaf samples were taken from three different individuals for each population, during the flowering and fruiting periods. The anatomical material was fixed in FAA (5 parts formaldehyde, 5 parts acetic acid, 90 parts 70% ethanol) for 24 hours and then stored in 70% ethanol (Johansen, 1940). Transverse and longitudinal sections were taken from the stems and leaves located between the fifth and sixth nodes from the woody base of the mature shoots using a Shandon Cryotome SME brand freezing microtome, following the method used by Güven et al. (2021). All the transverse and longitudinal sections were stained with hematoxylin, and mounted with aqua witrexia to prepare permanent slides, while the surface ones from the leaf blade were taken by free hand and examined from temporary slides. All the anatomical slides were examined and photographed using an Olympus BX51 light microscope (LM), and measurements related to the anatomical characters in Table 2 were made on the digital photographs using the Bs200Pro analysis system software.

2.3. Scanning electron microscopy

For SEM examinations, small pieces (1 cm x 1 cm) were cut superficially from the stem, petiole and lamina under a binocular microscope and dehydrated in ethanol series (50-80%). Afterwards, all the sections were critical-point dried with CO^2 using a Quorum K 850, and detailed images of the pubescence, stomata, epidermal cells, epicuticular wax and colleter structures on the surface of the vegetative parts, which are thought to be important in the distinction of taxa, were taken from the sections using a JEOL-JSM 6610 SEM microscope at the Recep Tayyip Erdoğan University Central Research Laboratories. In SEM examinations, the procedure used by Güven (2025a) was followed for sample preparation and examination, and the terminology used by Stearn (1992), Barthlott et al. (1998), Koch et al. (2008) and Prüm et al. (2012) was followed for the description of micromorphological characters.

2.4. Cytogenetic procedure

Somatic chromosomal examinations were perfomed on 10 mature seeds per each population, which were germinated on wet filter paper in Petri dishes at +25-28°C. 1–1.5 cm long root tips were cut and fixed in α-monobromonaphthalene at +4°C for 16 hours and in Carnoy solution (3:1 absolute alcohol:glacial acetic acid) overnight, respectively, then stored in 70% alcohol.

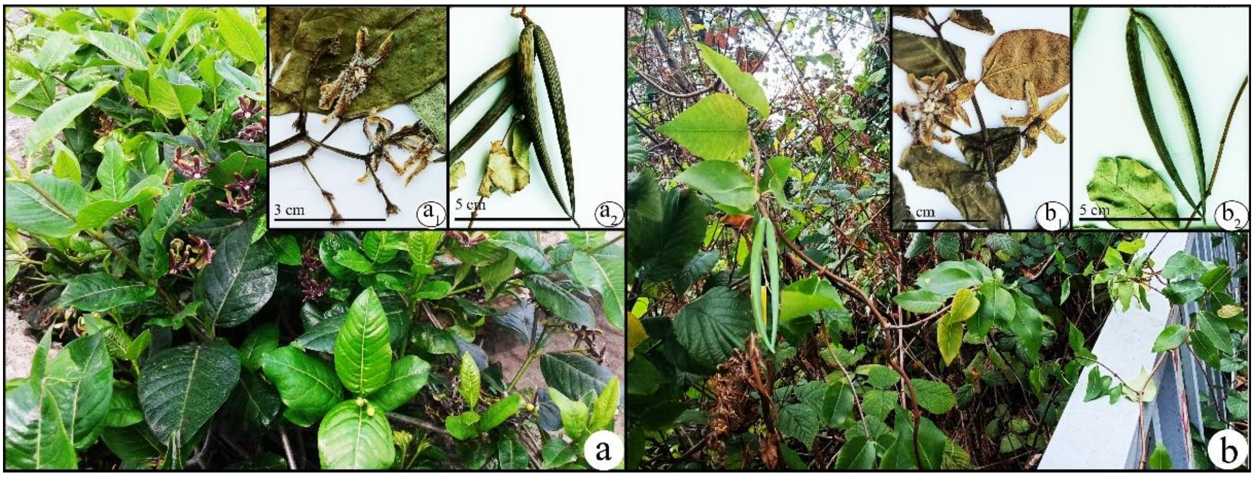

Figure 1. Examined *Periploca graeca* specimens: a. S. Güven 206, b. S. Güven 207, a_1 and b_1: Flowers, a_2 and b_2: Follicles.

Table 1. Annual climate data for Kastamonu and Trabzon (Measurement period: 1959-2024) (MGM, 2025).

Locality	Average Temp. (°C)	Max. Temp. (°C)	Min. Temp. (°C)	Average Sunshine Duration (hours)	Average Number of Rainy Days	Average Total Annual Rainfall (mm)
Kastamonu	9.9	42.2	-26.9	5.8	124.5	485.1
Trabzon	14.8	38.2	-7.4	4.5	140.2	829.1

Table 2. Anatomical characters of *Periploca graeca* taxa used in univariate comparative statistical analyses (*, statistically significant)

Symbol	Anatomical characters
*X_1	Width of periderma in the stem (µm)
*X_2	Width of collenchyma in the stem (µm)
*X_3	Width of cortex in the stem (µm)
*X_4	Width of xylem in the stem (µm)
X_5	Width of vascular bundle in the stem (µm)
*X_6	Width of xylem / width of vascular bundle in the stem (µm/µm)
X_7	Stem radius (µm)
X_8	Width of vascular bundle / stem radius (µm/µm)
X_9	Width of collenchyma beneath the lower epidermis in the petiole (µm)
X_{10}	Width of collenchyma beneath the upper epidermis in the petiole (µm)
*X_{11}	Width of xylem in the petiole (µm)
X_{12}	Width of vascular bundle in the petiole (µm)
*X_{13}	Width of xylem / width of vascular bundle in the petiole (µm/µm)
*X_{14}	Width of collenchyma beneath the lower epidermis in the midrib (µm)
X_{15}	Width of collenchyma beneath the upper epidermis in the midrib (µm)
X_{16}	Width of xylem in the midrib (µm)
X_{17}	Width of vascular bundle in the midrib (µm)
X_{18}	Width of xylem / width of vascular bundle in the midrib (µm/µm)
X_{19}	Average row number of upper palisade parenchyma (number)
*X_{20}	Width of upper palisade parenchyma (µm)
*X_{21}	Width of spongy tissue (µm)
X_{22}	Width of mesophyll tissue (µm)
*X_{23}	Width of upper palisade tissue / width of mesophyll tissue (µm/µm)
X_{24}	Average number of lower epidermal stomata (1 mm^2) (number)
*X_{25}	Average number of lower epidermal cells (1 mm^2) (number)
*X_{26}	Stomatal index of lower epidermis (number)
*X_{27}	Width of lower epidermal stomata (µm)
X_{28}	Length of lower epidermal stomata (µm)
*X_{29}	Width of lower epidermal cells (µm)
*X_{30}	Length of lower epidermal cells (µm)
*X_{31}	Average number of upper epidermal cells (1 mm^2) (number)
*X_{32}	Width of upper epidermal cells (µm)
X_{33}	Length of upper epidermal cells (µm)

Afterwards, the root tips were hydrolyzed with 1 N HCl for 10-12 minutes at 60°C, stained with 2% aceto orcein for 24 hours at room temperature, squashed in a drop of 45% acetic acid the preparations, and mounted in entellan in order to obtain permanent slides. The plates including metaphase chromosomes were photographed with an Olympus BX51 microscope, and chromosomes were counted on 10 × 100 enlarged photographs (Elçi, 1994; Martin et al., 2012).

2.5. Statistical Analysis

Quantitative anatomical data were obtained from two natural populations of *P. graeca* (Kastamonu and Trabzon), with 15 replicates per population and a total of 33 anatomical characters (X_1–X_{33}; Table 2). Descriptive statistics were calculated for each character and are presented as mean ± standard deviation (SD). Prior to comparative analyses, data normality for each character was assessed using the Shapiro–Wilk test. Characters showing a normal distribution were analyzed using the independent samples t-test, whereas characters deviating from normality were evaluated using the non-parametric Mann–Whitney U test. All statistical analyses were performed as two-tailed tests, and statistical significance was assessed at the 95% confidence level ($p < 0.05$). Characters with p values greater than 0.05 were considered not statistically significant (NS). In cases where $p = 1.000$, the two populations exhibited identical distributions for the respective character. All statistical analyses were performed using IBM SPSS Statistics.

3. Results

3.1 Anatomical description of the stem, petiole and lamina

In this study, detailed stem and leaf anatomy, supported by light microscopy and SEM images, was described for *P. graeca* using the plant individuals from Kastamonu and Trabzon populations. Anatomical measurements and evaluations are presented comparatively for both populations in Table 3.

In cross-section, the stem is circular in outline, and surrounded by a distinct peridermis composed of 6-7 layers of rectangular, thick-walled, and occasionally fragmented cells. Toward the interior of the ground tissue, 1-2 layered collenchyma and a multi-layered parenchyma including small groups of thick-walled sclerenchymatous fibers, are located, respectively. Bicollateral vascular bundles, surrounding the pith region in a continuous ring, comprise a distinct cambium between the external phloem and the xylem, and the internal phloem arranged in small groups adjoining the xylem. The external phloem contains dense druse crystals, and a small number of thick-walled sclerenchyma cells at the cortex border. The center is composed of a parenchymatic pith, and both cortex and pith tissues contain druse crystals and laticifers (Fig. 2a-f).

In cross-section, the petiole is circular in outline with small ridges at the both edges of the adaxial side (Fig. 2g, j), and the midrib is circular in shape (Fig. 3a, d). Both the petiole and midrib contain a main arc shaped bicollateral bundle in the center which is surrounded by multi-layered and thin-walled parenchyma cells, and 2-10 layered collenchyma cells beneath the adaxial and abaxial sides. Within the cortex parenchyma cells, idioblasts containing druse crystals and laticifers are clearly seen in both cross and longitudinal sections of the petiole and midrib (Fig. 2, 3). Many laticifers are observed throughout the midrib, petiole, and stem (Fig. 2, 3). Non-articulated laticifers are clearly seen in the longitudinal sections of the stems (Fig. 2c, f) and petioles (Fig. 2i, l).

The leaf is bifacial comprising single layer of palisade parenchyma beneath the upper epidermis and a multi-layered spongy parenchyma beneath the lower epidermis, and includes small vascular bundles in regular rows and idioblasts containing druse crystals throughout the mesophyll tissue (Fig. 3c, 3f). In superficial sections, the hypostomatic leaf contains anomocytic and paracytic stomata on the abaxial surface (Fig. 3h-i, 3k-l).

3.2. Micromorphological description of the stem, petiole and lamina

Colleters are located in groups of 3-8 at the base of the petioles of young leaves near shoot apices (Fig. 4a, d). All the petiolar colleters are described as the standard type, which have a conical, undivided main secretory head placed on a short stalk at the non-secretory basal portion (Fig. 4b, e).

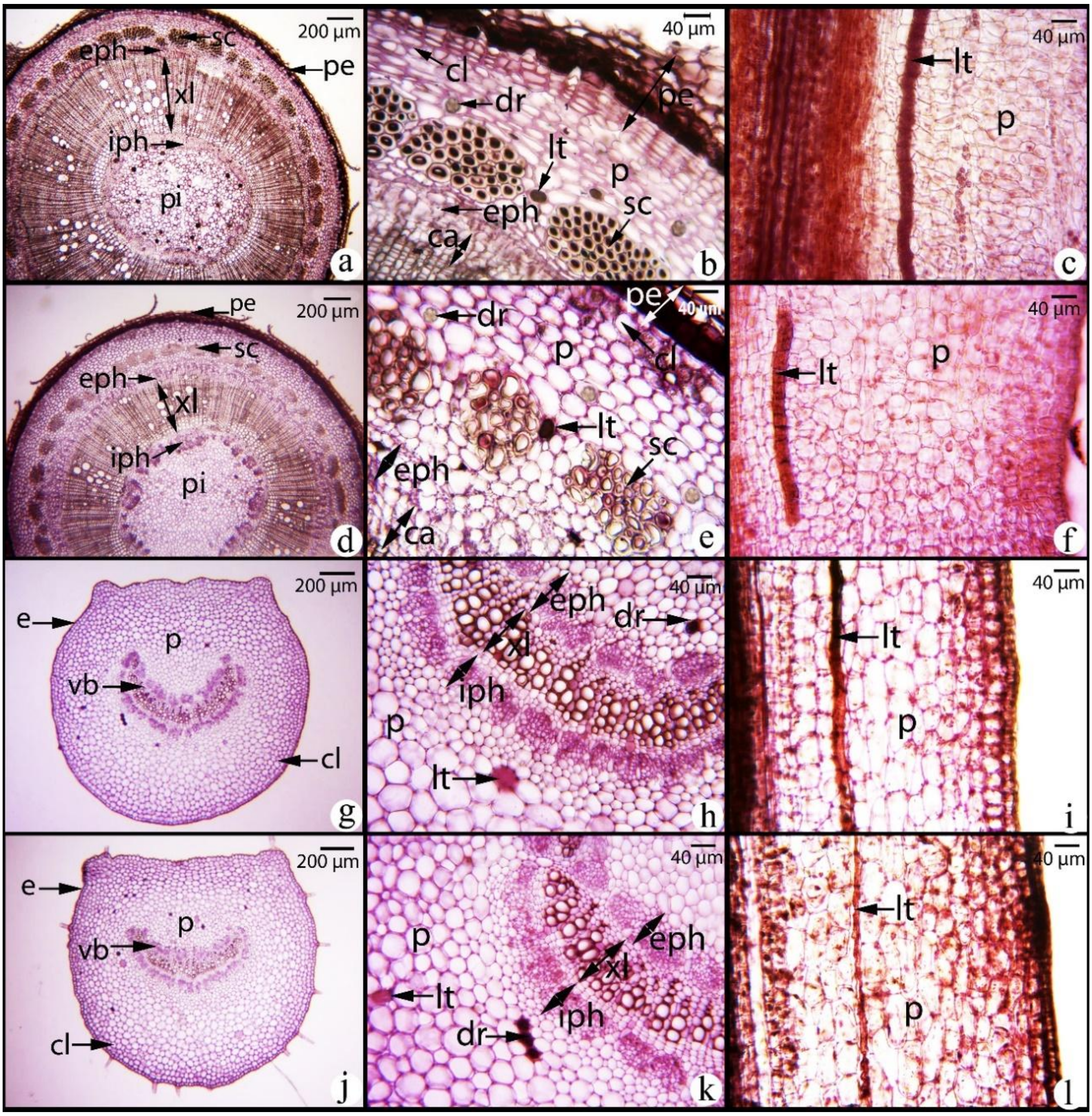

Figure 2. Stem and petiole anatomy of *Periploca graeca*: a-f. Transverse and longitudinal sections of the stem (a-c: S. Güven 206, d-f: S. Güven 207), g-l. Transverse and longitudinal sections of the petiole (g-i: S. Güven 206, j-l: S. Güven 207). ca: cambium, cl: collenchyma, e: epidermis, eph: external phloem, dr: druse, iph: internal phloem, lt: laticifer, p: paranchyma, pe: peridermis, pi: pith parenchyma, sc: sclerenchyma cells, vb: vascular bundle, xl: xylem.

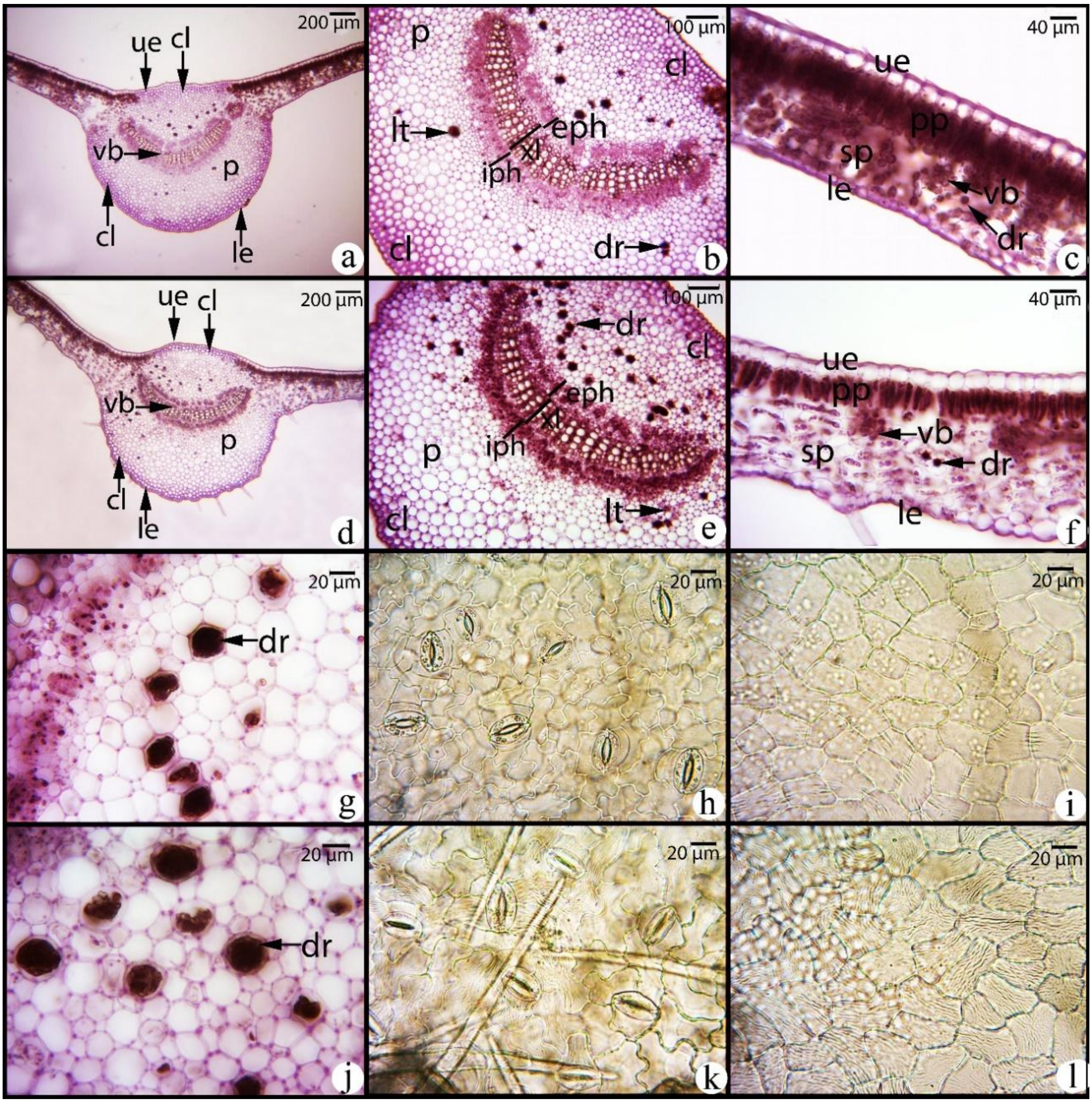

Figure 3. Leaf anatomy of *Periploca graeca*: a-f. Cross-sections of the midrib and mesophyll (a-c: S. Güven 206, d-f: S. Güven 207), g and j. Druse crystals in midrib (g: S. Güven 206, j: S. Güven 207), h and k: Superficial sections of the lower leaf surface (h: S. Güven 206, k: S. Güven 207), i and l. Superficial sections of the upper leaf surface (i: S. Güven 206, l: S. Güven 207). cl: collenchyma, dr: druse, eph: external phloem, iph: internal phloem, le: lower epidermis, lt: laticifer, p: paranchyma, pp: palisade paranchyma, sp: spongy paranchyma, ue: upper epidermis, vb: vascular bundle, xl: xylem.

Epicuticular wax layer is rough on the stem and petiole surface in the both examined populations (Fig. 4). The petiole is observed as glabrous, or with very sparse, short trichomes towards the tip, in the members of Kastamonu population (Fig. 4g-i), while the petiole surface is densely covered with villous indumenta of nonglandular, long, soft, straight trichomes in the individuals from Trabzon population (Fig. 4j-l).

The leaf is hypostomatic with paracytic and anomocytic stomata; epidermal cells on both adaxial and abaxial sides are polygonal with convex and concave periclinal cell walls and channelled, undulate anticlinal cell walls, epicuticular wax is striate for the both examined populations (Fig. 5) .

The leaf is almost glabrous or has very sparse, short trichomes towards the tip in the plant specimens from Kastamonu population (Fig. 5a-f). Whereas leaf surface is densely covered with villous indumenta of nonglandular, long, soft, straight trichomes on the abaxial side and with sparsely villous indumenta on the adaxial side in the individuals from Trabzon population (Fig. 5g-l).

3.3. Chromosome numbers

The somatic chromosome numbers were determined as 2n=2x=22 in the examined *P. graeca* specimens from both populations (Fig. 6).

3.4. Statistical Analysis

Comparative statistical analyses revealed that 18 out of 33 quantitative anatomical characters exhibited statistically significant differences between the Kastamonu and Trabzon populations (Table 3). The most efficient characters included tissue thickness–related traits such as the width of periderm (X_1), collenchyma (X_2) and cortex (X_3) in the stem, and the width of xylem (X_4, X_{11}), as well as the xylem to vascular bundle ratio (X_6, X_{13}) in the stem and petiole, the width of collenchyma in the abaxial side of midrib (X_{14}), the width of palisade parenchyma (X_{20}) and spongy parenchyma (X_{21}), and the palisade to total mesophyll ratio (X_{23}), the size and density of stomata on the abaxial leaf surface (X_{26}-X_{27}), the size and density of epidermal cells on the both leaf surfaces (X_{29}-X_{32}). In contrast, several characters related to general organ dimensions and internal tissue proportions, such as overall stem and leaf thickness (X_5, X_7-X_9), selected vascular traits (X_{10}, X_{12}, X_{15}–X_{18}, X_{22}, X_{24}), and surface-related features (X_{28}, X_{33}), did not differ significantly between populations (Table 3). Character X_{19} showed constant values across both populations and was therefore excluded from statistical evaluation.

4. Discussions

In the literature, very few information is available on the anatomy and micromorphology of Periplocoideae, including *P. angustifolia* (Dghim et al., 2015) belonging to the genus *Periploca*, and several taxa of the other genera of the subfamily as *Tacazzea* (Venter et al., 1990a) and *Petopentia* (Venter et al., 1990b). Although wood anatomy of *P. graeca* was investigated by Lens et al. (2009), the present study is the first comprehensive investigation on primary stem and leaf anatomical characteristics within the species. The examined two *P. graeca* taxa, which were collected from natural populations growing in different climatic and environmental characteristics in Türkiye, were found to be consistent in the following anatomical characters: stems which were surrounded by a distinct peridermis and contained small groups of thick-walled

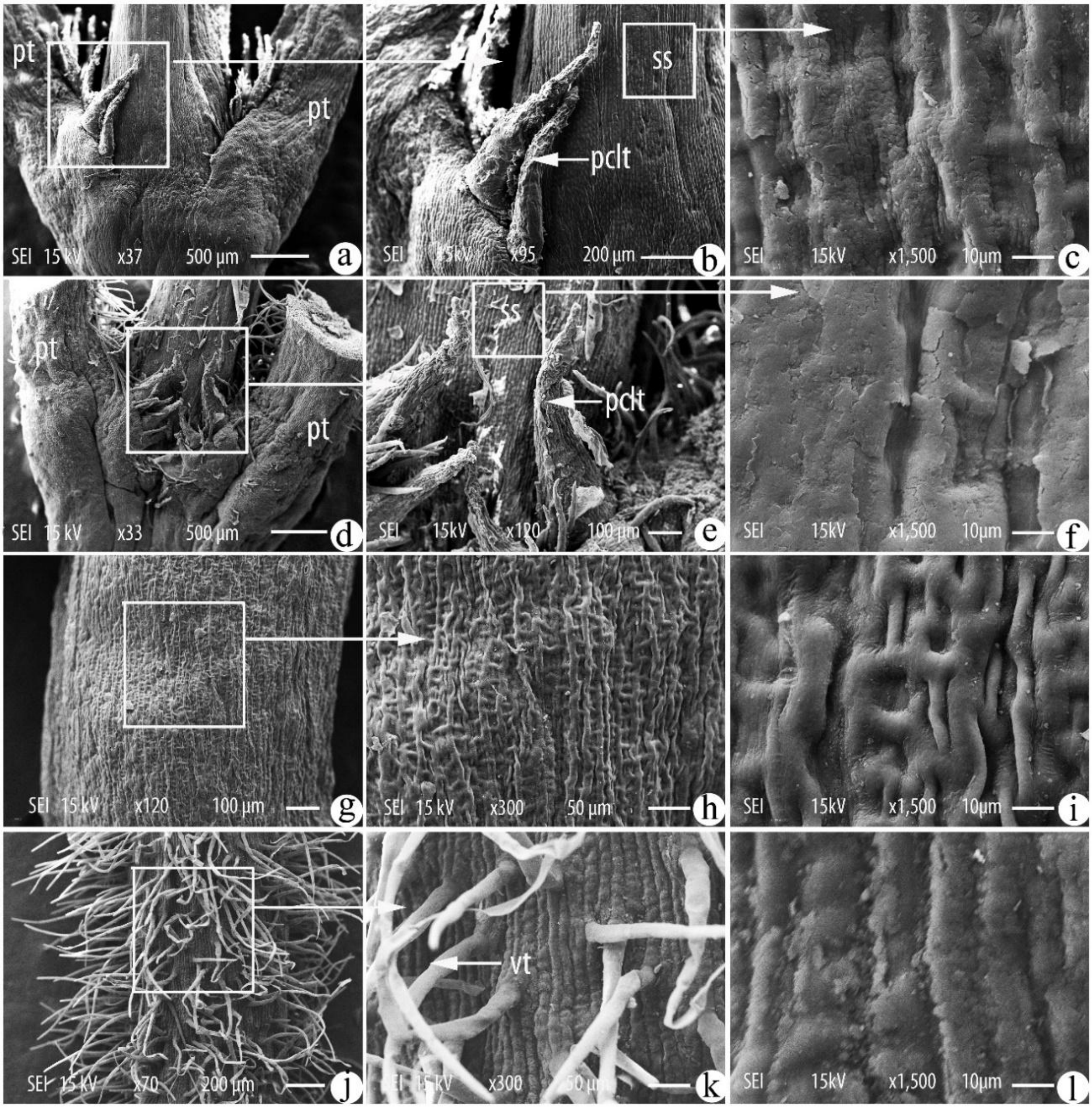

Figure 4. Scanning electron microscope images of stem and petiole surfaces of *Periploca graeca*: a,b,d,e. Petiolar colleters (a-b: S. Güven 206, d-e: S. Güven 207), c and f. Stem surface (c: S. Güven 206, f: S. Güven 207), g-l. Petiole surface (g-i: S. Güven 206, j-l: S. Güven 207). pclt: petiolar colleter, pt: petiole surface, ss: stem surface, vt: villous trichome.

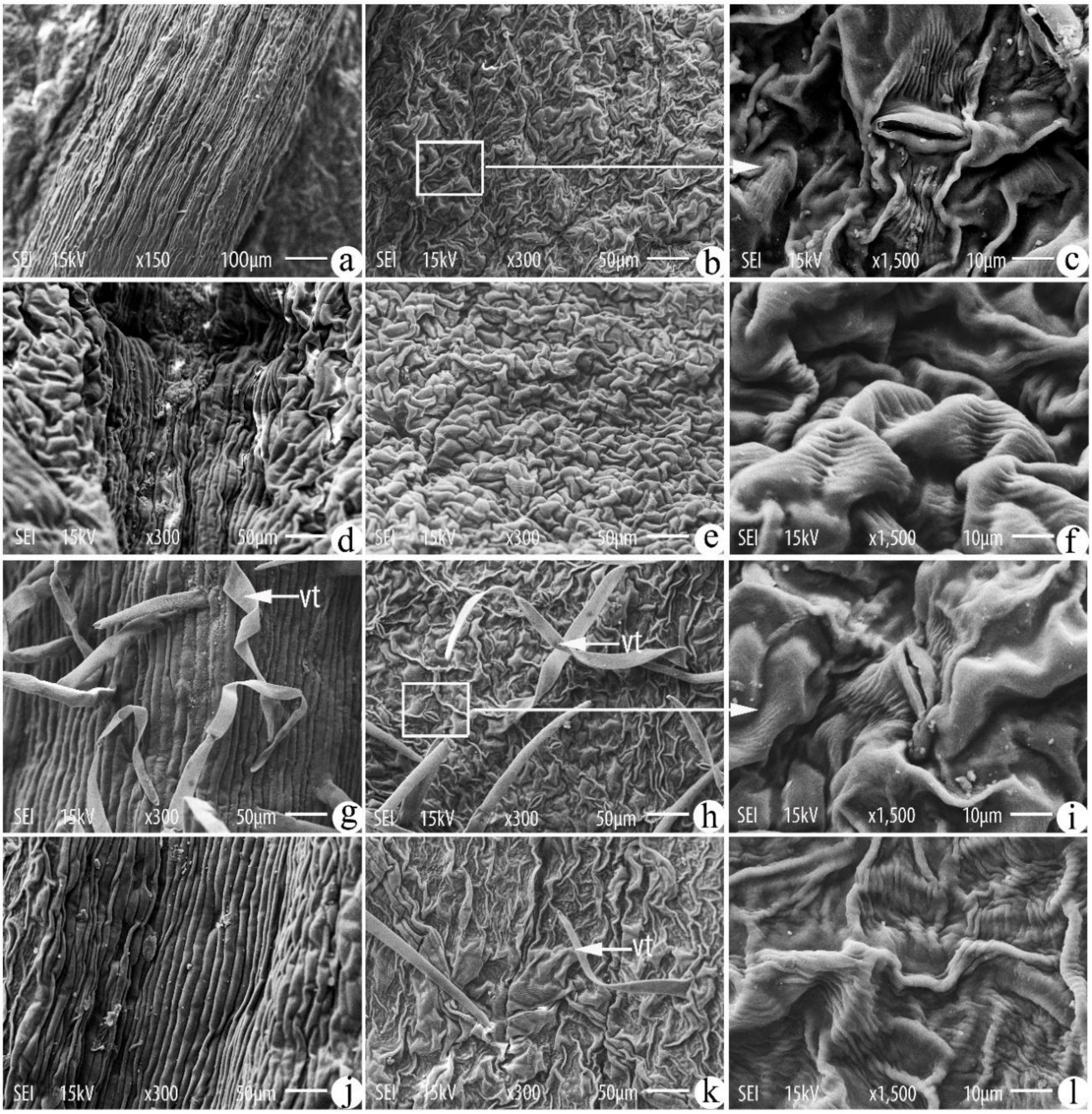

Figure 5. Scanning electron microscope images of lamina surface of *Periploca graeca*: a-c. Abaxial surface of the lamina of S. Güven 206, d-f. Adaxial surface of the lamina of S. Güven 206, g-i. Abaxial surface of the lamina of S. Güven 207, j-l. Adaxial surface of the lamina of S. Güven 207. vt: villous trichome

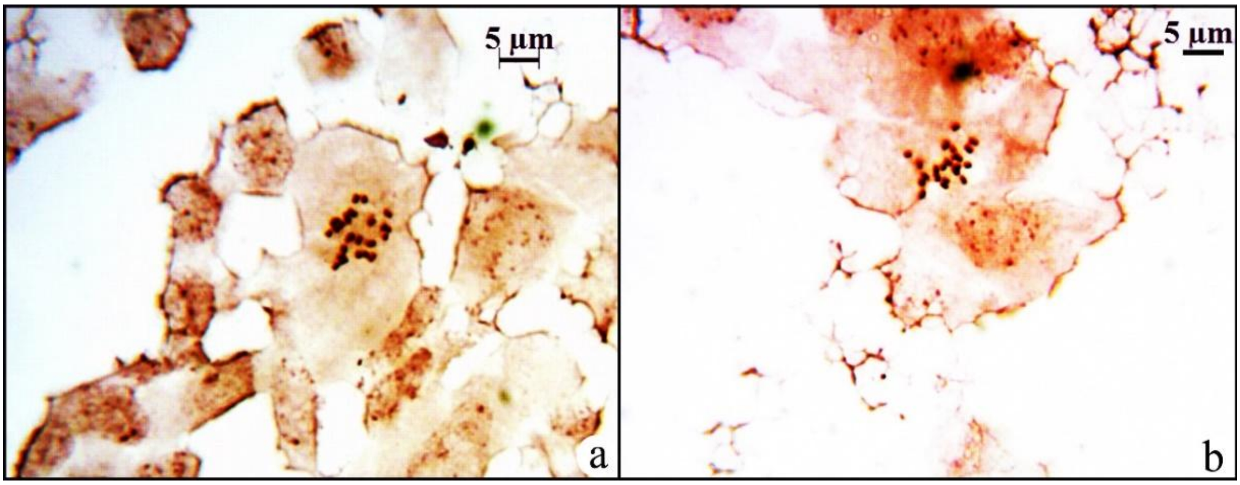

Figure 6. Somatic metaphases of *Periploca graeca*: a. 2n=22 (S. Güven 206), b. 2n=22 (S. Güven 207).

sclerenchymatous fibers located in the ground tissue, bifacial and hypostomatic leaves with anomocytic and paracytic stomata, and bicollateral vascular bundles, druse crystals and laticifers throughout the plant. The ability to adapt to various ecological conditions has led to the development of family-specific anatomical structures for the *Apocynaceae* taxa, as determined here for *P. graeca*, such as the presence of sclerenchymatous fibers in the stem, intraxylary phloem, crystals, and excretory tissues in the form of laticifers, resulting in the accumulation of various metabolites throughout the plants (Metcalfe and Chalk, 1950; Venter et al., 1990a, 1990b; Lens et al., 2009; Dghim et al., 2015).

The investigated *P. graeca* taxa exhibited similar anatomical structure by means of stems, including cortical sclerenchymatous fiber groups scattered along bicollateral vascular bundles and collenchyma cells beneath the epidermis, and petiole and midrib regions, comprising a main arc shaped bicollateral bundle in the center part surrounded by parenchyma cells and collenchyma cells beneath the epidermis. These results are consistent with the stem-leaf anatomy previously reported for another *Periploca* taxon, *P. angustifolia* (Dghim et al., 2015), several taxa belonging to the other Periplocoideae genera, *Tacazzea* (Venter et al., 1990a), *Petopentia* (Venter et al., 1990b), and some Turkish *Asclepiadoideae* members such as *Araujia* (Güven, 2026), *Cionura* (Güven, 2025a) and *Vincetoxicum* (Güven, 2025b), like the majority of the *Apocynaceae* (Metcalfe and Chalk, 1950). In primary structure of climbing plants, fibers in the stem cortex, and collenchyma at the outside of the vegetative parts have been considered the common anatomical structures providing mechanical support in the lianoid development (Lopes et al., 2008).

In the present numerical analyses (Table 3) several stem-related characters were found to have statistically significant differences between populations, including periderm width (X_1), collenchyma width (X_2), cortex width

Table 3. Univariate statistical comparison of anatomical characters between the Kastamonu and Trabzon populations

Chr.	Kastamonu (Mean ± SD)	Trabzon (Mean ± SD)	Test	Test statistic	p value	Result
X1	84.93 ± 8.49	93.27 ± 10.95	MWU	U=42.50, Z=−2.91	0.004	S
X2	32.67 ± 3.56	22.40 ± 4.40	t	t(28)=7.02	<0.001	S
X3	250.40 ± 31.17	323.20 ± 27.91	t	t(28)=−6.74	<0.001	S
X4	574.40 ± 105.14	478.67 ± 62.66	MWU	U=54.50, Z=−2.41	0.016	S
X5	730.40 ± 93.06	717.93 ± 84.17	t	t(28)=0.39	0.703	NS
X6	0.78 ± 0.07	0.67 ± 0.03	t	t(18)=5.91	<0.001	S
X7	1542.67 ± 114.31	1603.60 ± 67.18	t	t(23)=−1.78	0.089	NS
X8	0.47 ± 0.03	0.45 ± 0.04	t	t(28)=1.96	0.061	NS
X9	90.93 ± 10.87	82.87 ± 16.41	t	t(28)=1.59	0.124	NS
X10	117.73 ± 25.50	120.80 ± 22.94	MWU	U=93.50, Z=−0.79	0.430	NS
X11	95.93 ± 6.57	107.67 ± 12.57	t	t(28)=−3.20	0.003	S
X12	235.67 ± 10.98	244.07 ± 20.64	t	t(28)=−1.39	0.175	NS
X13	0.41 ± 0.03	0.44 ± 0.04	t	t(28)=−2.46	0.020	S
X14	83.73 ± 12.56	67.80 ± 11.92	MWU	U=45.00, Z=−2.80	0.005	S
X15	81.27 ± 16.66	73.13 ± 19.78	MWU	U=75.00, Z=−1.56	0.120	NS
X16	115.87 ± 12.39	116.47 ± 11.01	t	t(28)=−0.14	0.889	NS
X17	251.67 ± 20.45	257.00 ± 13.11	t	t(28)=−0.85	0.402	NS
X18	0.46 ± 0.02	0.45 ± 0.05	t	t(22)=0.45	0.660	NS
X19	1.00 ± 0.00	1.00 ± 0.00	MWU	U=112.50, Z=0.00	1.000	EX
X20	72.53 ± 8.48	51.27 ± 3.17	MWU	U=0.00, Z=−4.69	<0.001	S
X21	121.87 ± 21.39	135.87 ± 22.55	MWU	U=65.00, Z=−1.97	0.049	S
X22	195.60 ± 20.57	189.07 ± 23.77	t	t(28)=0.81	0.428	NS
X23	0.37 ± 0.05	0.27 ± 0.03	t	t(28)=6.48	<0.001	S
X24	128.89 ± 23.12	136.67 ± 12.91	MWU	U=96.00, Z=−0.72	0.470	NS
X25	1247.78 ± 145.41	964.44 ± 92.55	MWU	U=5.00, Z=−4.48	<0.001	S
X26	9.36 ± 1.41	12.47 ± 1.28	MWU	U=15.00, Z=−4.05	<0.001	S
X27	22.73 ± 1.28	20.90 ± 1.87	t	t(28)=3.13	0.004	S
X28	36.27 ± 1.83	37.97 ± 3.38	t	t(22)=−1.72	0.100	NS
X29	25.00 ± 6.44	29.65 ± 6.03	t	t(28)=−2.04	0.050	S*
X30	51.67 ± 8.72	63.86 ± 12.95	t	t(28)=−3.03	0.005	S
X31	1312.22 ± 64.69	964.44 ± 62.32	t	t(28)=14.99	<0.001	S
X32	25.20 ± 4.18	29.24 ± 4.69	MWU	U=50.00, Z=−2.60	0.009	S
X33	44.60 ± 8.46	48.90 ± 7.11	t	t(28)=−1.51	0.143	NS

Chr., character; Mean ± SD, mean ± standard deviation; MWU, Mann–Whitney U test; t, independent samples t-test; U, Mann–Whitney U statistic; Z, standardized test statistic; p, probability value; S, significant difference ($p < 0.05$); NS, not significant ($p \geq 0.05$); EX, excluded

(X_3), xylem width (X_4), and the xylem-to-vascular bundle ratio (X_6). It is known that these tissues are directly associated with mechanical support and hydraulic conductivity. The variation in stem anatomy between the two populations could be a result of the sensitives of the plants to local environmental conditions such as moisture availability, wind exposure, and mechanical stress. In particular, the significant change in xylem proportion (X_6) highlights adaptive differences in water transport efficiency between populations. Because xylem tissue is known to exhibit variable structure in response to water availability, with plants in drier environments typically developing proportionally increased xylem and narrower conductive elements to enhance hydraulic safety and reduce the risk of embolism (Tyree and Zimmermann, 2002; Hacke et al., 2006). Consistent with these results, populations in the Kastomunu region, which receives less rainfall, showed a wider xylem texture on their stems.

Mesophyll arrangement, stoma type and distribution are the most used foliar characters to delimite plant taxa (Coşkuncelebi et al., 2015). Leaves of the examined *P. graeca* taxa were bifacial with single layer of palisade parenchyma beneath the upper epidermis. Similar bifacial leaves were defined for some Periplocoideae taxa including *Tacazzea* (Venter et al., 1990a), *Petopentia* (Venter et al., 1990b) and *Raphionacme* (Venter & Verhoeven, 2000). However, mesophyll structure was observed as equifacial in *P. angustifolia* (Dghim et al., 2015). In many anatomical studies on the *Apocynaceae,* the mesophyll type (bifacial, monofacial, or isobilateral) have been used as distinguishing character at both the genus and species levels (El-Fiki et al., 2019; Bashir et al., 2020; El-Taher et al., 2020; Güven, 2025a,b, 2026). Although the two examined *P. graeca* taxa had similar mesophyll structure, significant differences were detected between populations in terms of palisade tissue thickness. In samples of *P. graeca* collected from Kastamonu, a region known for receiving less rainfall, higher temperature and longer sunshine suration during the vegetation period, compared to Trabzon (Table 1), palisade width and palisade width/mesophyll width ratio were measured to be higher (Table 2). Similarly, anatomical investigations on plants pointed out that sun leaves are smaller and thicker with higher palisade/spongy parenchyma ratio compared to shade leaves (Kim et al., 2005; Coşkuncelebi et al., 2015).

The studied *P. graeca* taxa exhibited hypostomatic leaves with anomocytic and paracytic stomata on the abaxial surface. Consistent with the present findings, several investigations regarding the members of both the subfamily Periplocoideae (Venter et al., 1990a,b; Venter and Verhoeven, 2000) and the other subfamilies in *Apocynaceae* (El-Fiki et al., 2019; Nisa et al., 2019; Bashir et al., 2020; El-Taher et al., 2020; Khan et al., 2025; Güven, 2025a,b, 2026) revealed that leaves were hypostomatic or amphistomatic which had mostly anisocytic stomata or in combination with various stomata types like anomocytic, anisocytic, cyclocytic, stephanocytic, brachyparacytic, and hemiparacytic. On the other hand, in populations growing in Trabzon, which has more humid climate receiving more rainfall during the vegetation period compared to Kastamonu, stomata density and size were determined to be higher. It is known that stoma characteristics are the most known foliar features varing greatly depending on plant species and environmental conditions. Similar to the present results, previous studies pointed out that plants grown in humid and temperate environments have larger and more numerous stomata compared to those grown in arid and hot environments (Driesen et al., 2020).

The statistical significance of several leaf mesophyll and epidermal characters in the present study, such as palisade parenchyma width (X_{20}), spongy tissue width (X_{21}), and the palisade-to-mesophyll ratio (X_{23}), stomatal index (X_{26}), stomatal size (X_{27}), and the size and number of epidermal cells on both leaf surfaces (X_{25}, X_{29}–X_{32}), is not unexpected, as these traits are well known to exhibit high phenotypic plasticity in response to environmental conditions. Taken together, the prominence of mesophyll and epidermal characters among the statistically significant traits supports the interpretation that the observed anatomical variation in *P. graeca* reflects environmentally driven anatomical plasticity across different habitats. Such variation, although statistically detectable, does not constitute reliable diagnostic evidence for infraspecific delimitation, but instead highlights the capacity of the species to adjust key anatomical features in response to local ecological conditions.

Presence of druse crystals in the stem, petiole and leaf structure of the examined *P. graeca* taxa is one of the most specific anatomical characters, which was previously reported for many *Apocynaceae* taxa (Metcalfe and Chalk, 1950; İlçim et al., 2010; El-Fiki et al., 2019; Sathya et al., 2022; Abeysinghe and Scharaschkin, 2022; Güven, 2025a,b, 2026). Calcium oxalate crystals were considered significant secondary metabolite supporting adaptation of *Apocynaceae* plants to extreme environmental conditions and resistance to herbivores and microbial invaders (Molik et al., 2025). Periplocoideae taxa are characterised by presence of milky latex in vegetative and generative parts (Endress and Bruyns, 2000). The laticiferous structure of *P. graeca* is presented in detail for the first time on cross-sections and longitudinal sections from the vegetative organs of two plant specimens from distinct habitats. Non-articulated laticifers were observed throughout the midrib, petiole, and stem structures of the examined taxa. Similarly, presence of non-articulated laticifers in the mesophyll and cortical parenchyma of stem was reported for *P. angustifolia* (Dghim et al., 2015). Laticifers, which were considered as important components of defense mechanisms within *Apocynaceae* (Agrawal and Konno, 2009), were traditionally described as non-articulated (Solereder, 1908; Metcalfe, 1967; Roy and De 1992; Serpe et al., 2001), as determined for *P. gracea* in the present study, or recently defined as articulated by several researchers (Demarco et al., 2006; Lopes et al., 2009, Güven, 2026). This study provided new contributions to the understanding of laticifer structure in *Apocynaceae,* which include very limited data related to laticifer. This research, which examined the anatomical and micromorphological structure of the vegetative parts of *P. graeca* taxa, revealed that secretory structures called "colleter" are located in groups of 3-8 at the base of the petioles. Each colleter is in standard type, which consists of a non-secretory stalk, and a conical, undivided secretory head, and structurally have a central axis covered with a palisade-like epidermis, as defined by Thomas (1991). These secretory structures, commonly found in *Apocynaceae* taxa, are taxonomically important characters used in species identification. Colleters which were found on various vegetative and generative structures such as nodes, leaf blades, petioles, cotyledons, bracts, bracteoles, calyx, and corolla, were considered as specific gland structures playing an important role in protecting developing meristems (Ribeiro et al., 2017). Dickison (2000) defined "colleters" as multicellular structures that produce an adhesive secretion protecting developing organs. In accordance with these findings, it has recently been determined that colleter structures located on the basis of leaf blade and petiole of some Turkish Asclepiadoideae taxa, such as *Cionura* (Güven, 2025a) and *Araujia* (Güven, 2026), were of the standard type.

The micromorphology of the stem and leaf surfaces of the examined *P. graeca* specimens was investigated for the first time, and it was determined that the epicuticular wax layer was rough on the surfaces of stem and petiole. Similarly, within the Turkish Asclepiadoideae, stem and petiole epicuticular wax layer was observed as rough in *A. sericifera* (Güven, 2026). However, in *Vincetoxicum* Wolf (Güven and Doğan, 2025) and *Cionura* (Güven, 2025a) stem and petiole surfaces were found to be smooth. In addition, epidermal cells on both adaxial and abaxial sides of the leaves were polygonal with convex and concave periclinal cell walls, channelled, undulate anticlinal cell walls, and cuticular wax structure comprising radial striations. Consistent with the current findings, leaf epidermal cells, exhibiting similar periclinal and anticlinal wall structure, were determined to have striated epicuticular wax in the Periplocoideae members as *Tacazzea* (Venter et al., 1990a), *Petopentia* (Venter et al., 1990b) and *Raphionacme* (Venter and Verhoeven, 2000), as well as in many taxa belonging to other subfamilies (Xin-sheng, 2010; Carvalho et al., 2017; Bashir et al., 2020; Güven and Doğan, 2025; Güven, 2025a, 2026). It is known that these striate ornamentations on the cuticle are a characteristic of xerophytic plants, protecting the plant from both water loss and harmful sun rays (Wilkinson, 1979; Alquini et al., 2003).

The most distinguishing characteristic for the examined two *P. graeca* specimens was determined as the micromorphological feature of the indumenta of petiole and leaf. While the petiole and leaf surface was densely covered with villous indumenta of nonglandular, long, soft, straight trichomes with verrucose ornamentation in the plant individuals from Trabzon population, the petiole and leaf was glabrous, or with very sparse, short trichomes towards the tip, in the members of Kastamonu population. Previous micromorphological examinations on members of *Apocynaceae* revealed that some taxa exhibited only multicellular non-glandular hairs (Güven and Doğan, 2025, Güven, 2025a), while others had both non-glandular and glandular hairs (Gabr et al., 2015; El-Taher et al., 2020; Medina et al., 2021). The structure, shape, and distribution of hairs, as well as surface ornamentation, have been used as distinguishing characters (Theobald et al., 1979). It was reported that several members of Periplocoideae (Venter and Verhoeven, 2000) and Asclepiadoideae (Liede, 1996; Carvalho et al., 2017; Güven and Doğan, 2025; Güven 2025a, 2026) revealed multicellular nonglandular hairs with warty surface, as determined here for the taxa of *P. graeca*.

This study provides the somatic chromosome number of 2n = 22 for the two examined *P. graeca* accessions from different localities in Türkiye for the first time. The present results are consistent with the previous counts for the European members of *P. graeca* reported by Darlington and Wylie (1955) and Kamari et al. (1994). However, 2n=24 chromosome number was reported for this species by Pardi (1933) and Lopane (1951). Ecological and geographical conditions where plants grow have been considered as main reasons for variations in chromosome numbers within the plant species (Morawetz, 1986). It was determined that plant taxa originating from distinct geographical regions could have different chromosome numbers (Ozcan et al., 2008). Although no difference in chromosome number was detected between these two taxa with different pubescence characteristics, this research has made a significant contribution to our knowledge about the chromosome number of *P. graeca*.

Conclusion

This study provides the first comprehensive anatomical, micromorphological and karyological data for *Periploca graeca* based on populations growing under different ecological conditions in Türkiye. Although infraspecific differentiation within *P. graeca* is no longer supported in current taxonomic treatments (Venter, 1997; Güven and Sarıkaya, 2024; POWO, 2026), individuals displaying contrasting leaf and petiole pubescence were intentionally selected in order to assess whether previously emphasized variable characters are associated with broader structural differences or reflect environmentally driven anatomical plasticity. In addition, individuals exhibiting dense leaf and petiole pubescence, previously reported from a limited number of localities in Türkiye, particularly from Samsun and Eskişehir (Browicz, 1978), were also documented in the eastern Black Sea region in the present study. This finding extends the known geographical occurrence of pubescent phenotypes within *P. graeca* and indicates that such traits are not geographically restricted but may occur across a broader range of habitats. The presence of pubescent individuals in different ecological settings further supports the interpretation that leaf and petiole pubescence represents an environmentally influenced and plastic character rather than a stable taxonomic marker.

As seen in Table 3, statistical analyses revealed significant population-based variation in a total of 18 anatomical traits related to the stem (X_1, X_2, X_3, X_4, X_6), leaf (X_{11}, X_{13}, X_{14}, X_{20}, X_{21}, X_{23}, X_{25}-X_{27}, X_{29}-X_{32}). Despite the statistical significance of these differences, the overall anatomical, micromorphological and karyological similarity observed between the examined populations indicates that the detected variation represents ecological and phenotypic plasticity rather than taxonomically meaningful discontinuities. The consistent chromosome number (2n = 22) and the shared structural organization of vascular tissues, laticifers, and glandular structures further support this interpretation. Consequently, the present results demonstrate that environmentally responsive characters, including pubescence-related traits, are insufficient for delimiting infraspecific taxa within *P. graeca*, and instead document adaptive anatomical variation across different habitats. These findings reinforce the current species-level treatment of *P. graeca* and contribute valuable baseline data for understanding intraspecific structural diversity within the species.

Conflict of Interest

The author declares no conflict of interest.

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