Comparative Morphological and Anatomical Studies on *Morus* Species (Moraceae) in Turkey

Türkiye’deki *Morus* Türleri (Moraceae) Üzerinde Karşılaştırmalı Morfolojik ve Anatomik Çalışmalar

**Abstract**

Objectives: *Morus alba* L., *Morus nigra* L., and *Morus rubra* L. are widely cultivated in many countries due to their nutritive, economic, and medicinal value. In this study, comparative morphological and anatomical studies on three common *Morus* L. species found in Turkey were carried out. According to the results, differences regarding the morphological and anatomical features of these species were described, and the data were displayed in detailed photographs.

Materials and Methods: Specimens collected from different provinces of Turkey were studied. In the anatomical studies, investigations were performed on transversal and superficial sections of the leaves. All sections were stained with chloral hydrate and Sartur solution and were then examined using an Olympus BH2 light microscope.

Results: Significant diagnostic characteristics were found, such as trichome types, stomatal measurements, the stomatal index, and the density ratio of the parenchyma and collenchyma layers. Some morphological features of the leaves also showed prominent differences.

Conclusion: Our results may contribute to the taxonomy of *Morus* species for future work and be helpful in species diagnosis.

Key words: Moraceae, *Morus*, anatomy, morphology, Turkey

**Öz**

Amaç: *Morus alba* L., *Morus nigra* L. ve *Morus rubra* L. türleri gıda, ekonomik ve tıbbi değerleri nedeniyle birçok ülkede yaygın olarak yetiştirilmektedir. Bu çalışmada, Türkiye’de bulunan *Morus* L. türleri üzerinde karşılaştırmalı morfolojik ve anatomik incelemeler yapılmıştır. Sonuçlara göre, türlerin morfolojisini ve anatominin özellikleri ile ilgili farklılıklar tanınmıştır ve elde edilen veriler detaylı fotoğraflarla gösterilmiştir.

Gereç ve Yöntemler: Türkiye’nin farklı illerinden toplanan örnekler çalışılmıştır. Anatomik çalışmalarında, yaprakların enine ve yüzey kesitleri üzerinde incelemeler gerçekleştirilmiştir. Tüm kesitler kloralhidrat ve Sartur çözeltisi ile boynmuş ve ardından Olympus BH2 ışık mikroskobu kullanılarak incelenmiştir.

Bulgular: Trikom tipleri, stomatal ölçümleri, stomal indeksi, parenkima ve kolencoğum tabakalarının yoğunluk oranları gibi belirgin diagnostik karakterler bulunmuştur. Yapıların bazı morfolojik özellikleri de belirgin farklılıklar göstermiştir.

Sonuç: Sonuçlarımız, gelecek çalışmalar için *Morus* türlerinin taksonomisine katkıda bulunabilir ve türlerin ayırt edilmesinde yardımcı olabilir.

Anahtar kelimeler: Moraceae, *Morus*, anatomi, morfoloji, Türkiye

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INTRODUCTION

The genus Morus L. belongs to the Moraceae (mulberry) family, which contains 37 genera and nearly 1,100 species distributed throughout tropical and temperate regions worldwide. Morus species are generally known as mulberries, and their distribution is extensive in the East, West, and South East Asia, South Europe, the South of North America, the Northwest of South America, and some parts of Africa. It can be said that they have a high adaptation capacity for various environmental conditions. Mulberries are under cultivation in many different world regions, such as tropical, subtropical, and temperate zones of Asia, Europe, North and South America, and Africa. These species have economic value in most countries because of their use in sericulture. Moreover, they have been widely used as traditional folk medicine, particularly in China and India.

Mulberries are grown for the production of edible fruits in other countries like Turkey and Greece. They have a long history of cultivation, having been cultivated as food plants for more than 400 years in Turkey, one of the most important centers of diversity. In Turkey, the best known species are black mulberry (Morus nigra L.), white mulberry (Morus alba L.), and purple mulberry (Morus rubra L.). Besides the traditional medicinal use of various parts of these species, their fruits are also used in making syrup, jam, pulp, ice-cream, vinegar, and natural dyes. Flavonoids, anthocyanin and alkaloids contained in the most parts of mulberries ensure several pharmacological activities such as antidiabetic, antioxidant, antiinflammatory, antimutagenic, anticarcinogenic, and hepatoprotective properties.

According to the APG IV classification system, the family Moraceae belongs to the order Rosales within the Rosids clade. Diagnostic indicators of Moraceae include the presence of milky latex, a distinct stipule, anatropous ovules, apical placentation, compound fruits (achenes or syconous), and a cystolith. The genus Morus has also attracted the attention of many researchers due to its interesting breeding properties. Several studies have been performed on the morphology of Morus species throughout the world. Despite certain morphological differences, sometimes fruits of M. nigra and M. rubra may not be identified by local people, and sellers replace M. nigra with an less expensive black fruit. Moreover, some taxa show minor differences in leaf morphology. Anatomical studies of leaves provide many important diagnostic characteristics, such as the size, shape, and orientation of stomata, guard cells, and subsidiary cells; type and shape of trichomes; and structure of epidermal cells. For these reasons, determining the morphological and anatomical differences between species could be helpful in resolving diagnostic challenges.

Several studies have been performed on the morphology and anatomy of Morus. However, the leaf anatomy and morphology of Morus species from Turkey have not been investigated. The present study aims to investigate the morphological and anatomical features of M. alba, M. nigra, and M. rubra distributed in Turkey. We also attempted to identify diagnostic anatomical and morphological properties that could contribute to the taxonomy of the genus.

Experimental

Herbarium specimens were used to determine morphological and anatomical properties. M. alba collected from Balıkesir [Herbarium of Istanbul University Faculty of Pharmacy (ISTE) 109772] and Istanbul (ISTE 116445), M. nigra collected from Istanbul (ISTE 40073), and M. rubra collected from Gaziantep (ISTE 40076) are stored in the ISTE. Collected data on each studied species are shown in Table 1. Morphological studies were carried out on herbarium materials. For anatomical studies, leaves were pretreated by immersion in warm water. Minimum 15 individual specimens were used. Hand sections were taken from samples with a razor blade then stained with chloral hydrate and Sartur solution. Sections were examined using an Olympus BH2 light microscope, and detailed photos were taken using a Canon Power Shot A640 camera. Measurements of each samples were performed with KAMERAM® software, and the obtained data are given below. The stomatal index (SI) was calculated according to the following formula: SI: (S/S + E) x100, where S refers to the number of stomata per unit area, and E to the number of epidermal cells in the same unit area. No further statistical analysis was used.

| Table 1. Collection data of Morus taxa examined |
|------------------------------------------------|
| Taxon             | Locality, voucher number (ISTE)                        |
| Morus alba       | B1 Balıkesir: Kepsut, Büyükkatranç village, 800 m elevation, 30.05.2015, ISTE 109772 |
| Morus nigra      | A2 (E) İstanbul: Çatalca, İnceçiz gateway, field border, 50 m elevation, 25.05.2003, ISTE 80737 |
| Morus rubra      | C6 Gaziantep: Between Nizip-Gaziantep, Altindağ village, 650 m elevation, 30.05.1978, ISTE 40076 |

ISTE: Herbarium of Istanbul University Faculty of Pharmacy
RESULTS AND DISCUSSION

The lamina anatomical traits of the collected specimens were defined by examination of the lamina transverse and surficial sections.

*Morus alba* L.

The midrib is rich in collenchymatic elements with 4-5 layered collenchyma located under the lower epidermis, 2-3 layered collenchyma are located under the upper epidermis (Figure 1A-G). Between 5 and 6 layers of parenchyma cells exist between the collenchyma layers and the collateral vascular bundles (Figure 1A). The average leaf thickness at the midrib is 735.105 µm. Moreover, plenty of druse crystals of calcium oxalate were observed in the midrib and the mesophyll of the leaf (Figure 1C, F, I). Prismatic crystals were observed only in the midrib region. The density of the crystals is increased near the veins in the midrib (Figure 1C).

There is a single layered epidermis covered with a thin cuticle on both adaxial and abaxial surface of the lamina. The epidermis has polygonal cells with usually straight anticlinal

Figure 1. The transverse and surface sections of the leaf of *Morus alba*. The midrib region (A–H), mesophyll (I), adaxial surface (J), and abaxial surface (K) co: Collenchyma, dr: Druse, g: Peltate gland, le: Lower epidermis, ph: Phloem, pp: Palisade parenchyma, pr: Parenchyma, prc: Prismatic crystal, sp: Spongy parenchyma, st: Stomata, t: Non-glandular trichome, ue: Upper epidermis, xy: Xylem, vb: Vascular bundle
walls, and the upper epidermis cells are larger than the lower ones. The length and width of epidermis cells are presented in Table 2. Unicellular non-glandular trichomes, varying in length, are also present on both leaf surfaces. The number of non-glandular trichomes is higher along the veins and the midrib (Figure 1A, H). Glandular trichomes with a unicellular stalk and multicellular head are sparse on the lower surface. The leaf is dorsiventral. The mesophyll is composed of two layers of palisade cells under the upper epidermis and 5-6 layers of spongy cells under the lower epidermis. Cylindrical palisade cells were found in the transverse section. Spongy parenchyma cells with wide intercellular spaces have ovoid or circular shapes (Figure 1I). The spongy parenchyma occupies about 60.57% of the mesophyll. Stomata cells were found only on the abaxial surface of the leaf (hypostomatic) (Figure 1J, K). The leaf blade thickness ranges from 159.096 to 175.017 µm, with a mean value of 169.112 µm.

On the abaxial surface, anomocytic stomata are oval shaped and vary in size. They are situated at the same level as the other epidermal cells (mesomorphic). Each stoma is surrounded by 5-6 subsidiary cells (Figure 1K). Lithocysts, a specific type of enlarged epidermal cells in which calcium carbonate is deposited, and peltate glands were detected on the upper surface of the leaf (Figure 1J). The SI for the lower surface of the lamina was calculated as 10.71.

**Morus nigra L.**

Regarding the midrib region, 2-3 layered collenchyma are located under the lower epidermis, 1-2 layered collenchyma are located under the upper epidermis (Figure 3A-G). Parenchyma cells form 4-5 layers. They are present between the collenchyma layers and the collateral vascular bundles. The leaf thickness at the midrib is on average 740.899 µm. Many druse crystals of calcium oxalate were observed in the midrib and also in the mesophyll (Figure 2B, C, E, I, J). Several prismatic crystals were observed only in the midrib region. The crystals are abundant near the veins in the midrib (Figure 2B, C).

Both leaf surfaces have a single layered epidermis covered with a thin cuticle. Epidermis cells, which are polygonal in shape, usually have straight anticlinal walls. Their sizes are variable. The upper epidermal cells are larger than the lower ones (Table 2). Unicellular, non-glandular trichomes were observed on both leaf surfaces, and their number was higher on the lower surface (Figure 2A, D, G). Glandular trichomes with a unicellular stalk and multicellular head are scattered on both surfaces (Figure 2H). The mesophyll consists of two layers of palisade cells under the upper epidermis and 4-5 layers of spongy cells with wide intercellular spaces under the lower epidermis. Hence, the leaf is dorsiventral. While the palisade parenchyma cells were cylindrical, the spongy parenchyma cells were found to be ovoid-circular in transverse section (Figure 2I, J). The spongy parenchyma occupies approximately 64.46% of the mesophyll. The leaf is also hypostomatic and mesomorphic, stomata cells were found only on the lower surface of the leaf (Figure 2K-M). Leaf blade thickness ranges from 149.042 to 160.843 µm, with a mean value of 158.052 µm.

The stomata are anomocytic. They have an oval shape and vary in size. Each stoma is surrounded by 5-6 subsidiary cells (Figure 2L). Lithocysts and peltate glands were defined on the upper surface of the leaf (Figure 2I, K). The SI for the lower surface of the lamina was calculated as 13.26.

**Morus rubra L.**

In the midrib, while 2-3 layered collenchyma are located under the lower epidermis, 1-2 layered collenchyma are located under the upper epidermis (Figure 3A-G). Parenchyma cells form 4-5 layers. They are present between the collenchyma layers and the collateral vascular bundles. The leaf thickness at the midrib is on average 516.083 µm. Many druse crystals of calcium oxalate were observed in the midrib and also in the mesophyll (Figure 3B, C, F). Several prismatic crystals were observed only in the midrib region.

The epidermis cells, which are covered by a thin cuticula layer on both surfaces of the leaf, is single layered. Their shape is polygonal, and they vary in size (Table 2). However, the upper epidermis cells are larger than the lower epidermis cells. They usually have straight anticlinal walls. On both leaf surfaces, indumentum of unicellular non-glandular trichomes were noticed, but they were more numerous on the lower surface (Figure 3H, I). Glandular trichomes with a unicellular stalk and head were rare on both surfaces. The leaf is dorsiventral and

| Species       | Length x width (µm) | Length x width (µm) | Length x width (µm) |
|---------------|---------------------|---------------------|---------------------|
| **Morus alba**|                    |                    |                      |
| UEC           | 32.93 (23.62-44.06) | 20.14 (15.55-28.67) | 28.50 (37.81-40.42) |
| LEC           | 19.73 (13.13-29.40) | 18.25 (16.03-22.41) | 28.13 (26.95-30.18) |
| LS            | 18.99 (15.39-23.37) | 19.12 (16.71-21.19) | 34.22 (31.39-36.98) |
| PPL (thickness)| 56.139              | 40.081              | 45.008              |
| SPL (thickness)| 86.254              | 72.685              | 71.222              |

*UEC: Upper epidermis cell, LEC: Lower epidermis cell, LS: Stomata of abaxial epidermis, PPL: Palisade parenchyma layer, SPL: Spongy parenchyma layer, min: Minimum, max: Maximum*
hypostomatic (Figures 3J-L). The mesophyll is differentiated into palisade and spongy parenchyma. It comprises one layer of palisade cells under the upper epidermis and 3-4 layers of spongy cells with wide intercellular spaces under the lower epidermis. Palisade parenchyma cells are cylindrical, and the spongy parenchyma cells are ovoid-circular (Figure 3J). The spongy parenchyma occupies about 61.28% of the mesophyll. Leaf blade thickness ranges from 125.705 to 133.690 µm, with a mean value of 130.398 µm.

Figure 2. The transverse and surface sections of the leaf of *Morus nigra*. The midrib region (A-H), mesophyll (I, J), adaxial surface (K), and abaxial surface (L, M).

co: Collenchyma, dr: Druse, g: Peltate gland, gt: Glandular trichome, le: Lower epidermis, ph: Phloem, pp: Palisade parenchyma, pr: Parenchyma, prc: Prismatic crystal, sp: Spongy parenchyma, st: Stomata, t: Non-glandular trichome, ue: Upper epidermis, xy: Xylem, vb: Vascular bundle.
Oval shaped and different sized stomata are anomocytic. Each stoma is surrounded by 5-6 subsidiary cells, and the leaf is mesomorphic (Figure 3L). Lithocysts and peltate glands were found on the upper surface of the leaf (Figure 3K). The SI for the lower surface of the lamina was calculated as 11.11.

*Morus* species could vary in morphological appearance when the climate or habitat change. Hence, it is difficult to assign a taxonomic classification to these species. Comparative morphological and anatomical studies are the basic tools of plant taxonomy, and they provide fundamental data which are helpful for a majority of classification systems. Furthermore,

**Figure 3.** The transverse and surface sections of the leaf of *Morus rubra*. The midrib region (A-I), mesophyll (J), adaxial surface (K), and abaxial surface (L). co: Collenchyma, dr: Druse, g: Peltate gland, le: Lower epidermis, ph: Phloem, pp: Palisade parenchyma, pr: Parenchyma, prc: Prismatic crystal, sp: Spongy parenchyma, st: Stomata, t: Non-glandular trichome, ue: Upper epidermis, xy: Xylem, vb: Vascular bundle.
studies based on plant morphology and anatomy help us to understand the phylogeny of life. In this study, *M. alba*, *M. nigra*, and *M. rubra* were examined and compared morphologically and anatomically. Differences as a result of the investigation are given in Table 3.

Certain morphological characteristics, such as leaf shape, size, base, margin, and indumentum were found to be useful for identifying the studied species (Figure 4). Moreover, the indumentum of the shoot and the peduncle and the size of the fruit, peduncle, and petiole differ in these species. The species with the broadest leaves was found to be *M. alba*. The difference in the color of the fruits of these three species is perhaps the most striking organoleptic feature. *M. alba* has white, pinkish, or purplish fruit, whereas *M. nigra* has blackish-violet or black fruit, and *M. rubra* has dark reddish-purple fruit. Moreover, there are some similarities in their morphological features, such as inflorescences of short, dense spikes, ellipsoid syncarps, and fleshy drupelets.

We also know that some anatomical traits are very diagnostic. Thus, they are frequently used in routine identification. Since the leaf is regarded as the most varied organ of the angiosperms, taxonomic studies of various taxa were carried out on the basis of leaf anatomy. These studies present many anatomical characteristics of potential taxonomic significance. The

### Table 3. Morphological and anatomical comparison of the studied taxa

|                     | *Morus alba* | *Morus nigra* | *Morus rubra* |
|---------------------|--------------|---------------|--------------|
| **Shoots**          | Slender, glabrous | Stout, pubescent | Slender, pubescent |
| **Peduncle**        | Hairy        | Hairy         | Pubescent    |
| **Peduncle length** | (1-) 2 cm, circa as long as syncarp | 1-1.5 cm | 0.5-1 cm, 1/2 as long as syncarp |
| **Fruit length**    | (1-) 1.5-2.5 cm | (1.5-) 2-2.5 cm | (1.5-) 2-3 cm |
| **Fruit color**     | White, pinkish, or purplish | Blackish-violet or black | Dark reddish-purple |
| **Leaf shape**      | Ovate to broadly ovate | Broadly ovate | Broadly ovate to oblong-ovate |
| **Leaf size**       | 3-10 (-18)x2-12 cm | 5-12 (-20)x(4) -5.5-13 cm | 6-12 (-20)x4-10 cm |
| **Leaf apex**       | Acute or shortly acuminate | Acute or shortly acuminate | Abruptly long-acuminate |
| **Leaf base**       | Rounded or obliquely cordate | Deeply cordate | Truncate or subcordate |
| **Leaf margin**     | Crenate-dentate | Serrate | Serrate |
| **Indumentum of leaf** | Upper surface glabrous/lower surface pubescent on the midrib and the veins | Upper surface scabrous/lower surface pubescent | Upper surface slightly scabrous/lower surface roughly hairy |
| **Non-glandular trichomes** | Unicellular trichomes on the both leaf surfaces (density is higher along the veins and the midrib) | Unicellular trichomes on the both leaf surfaces (density is higher on the lower surface) | Unicellular trichomes on the both leaf surfaces (density is higher on the lower surface) |
| **Glandular trichomes** | Trichomes with unicellular stalk and multicellular head on the lower surface (sparsely) | Trichomes with unicellular stalk and multicellular head on the both surface | Trichomes with unicellular stalk and head on the both surfaces (rarely) |
| **Epidermal cells** | Upper epidermis cells are larger than the lower ones | Upper epidermis cells are larger than the lower ones | Upper epidermis cells are larger than the lower ones |
| **Mesophyll type**  | Dorsiventral | Dorsiventral | Dorsiventral |
| **Mesophyll**       | 38%-45% palisade parenchyma (2 layer) | 35%-38% palisade parenchyma (2 layer) | 35%-40% palisade parenchyma (one layer) |
| **Location of stomata** | Hypostomatic | Hypostomatic | Hypostomatic |
| **Stomatal index**  | 10.71 | 13.26 | 11.11 |
| **Collenchyma cell layers of midrib** | 4-5 layered on the lower surface, 2-3 layered on the upper surface | 2-3 layered on the lower surface, 1-2 layered on the upper surface | 2-3 layered on the lower surface, 1-2 layered on the upper surface |
| **Thickness of leaf blade (average)** | 169.112 µm | 158.052 µm | 130.398 µm |
| **Thickness of midrib (average)** | 735.105 µm | 516.083 µm | 740.899 µm |
| **Petiole length**  | 1-3.5 (-4) cm | 1.5-3.5 cm | (1-) 1.5-3 cm |
results of our detailed anatomical study revealed that there were some differences among the leaf anatomy of these three taxa. Metcalfe and Chalk\textsuperscript{25} have reported that the epidermis of the Moraceae generally comprises of a single layer of quadrangular or elongated anticlinal cells. Although the upper epidermis cells were found to be larger than the lower ones in all studied taxa, the length and width of epidermis cells on the two sides differed. Accordingly, the epidermis cells on the lower and upper surfaces of \textit{M. nigra} were found to be smaller than those in the other two taxa. Since the stomatal size may change according to environmental conditions, some authors do not regard this as a diagnostic characteristic. However, the stomatal size is generally accepted because the size of the stomata is generally stable enough to be used as a diagnostic characteristic.\textsuperscript{36,37} In the Moraceae family, stomata usually do not have special subsidiary cells.\textsuperscript{38,39} Leaves of the three taxa were determined to be hypostomata (stomata were only observed on the abaxial surface) with anomocytic-type stomata. However, concerning size, the width, and length of stomata were significantly different. The mean value of the stomata size of \textit{M. rubra} was found to be the highest species. The term SI is used to define stomatal frequency, and the size of the epidermal cells is neglected. Since taxa from distinct localities have more or less constant SI values, the SI is considered as a significant taxonomic characteristic.\textsuperscript{36,40} In the taxa studied, different values of the SI were calculated in this study.

Many studies have revealed the taxonomic value of trichomes in angiosperms.\textsuperscript{41,42} Glandular and non-glandular trichomes are common in the Moraceae.\textsuperscript{18,22,38} According to a previous study, while simple, unicellular, non-glandular trichomes and multicellular, capitate, glandular trichomes are common in \textit{Morus} taxa, conical unicellular non-glandular trichomes and bicellular capitate glandular trichomes are rare.\textsuperscript{38} Abbasi et al.\textsuperscript{26} indicated that unicellular non-glandular and glandular trichomes and also hooked hairs are present on the leaf surfaces of \textit{Morus} species. Moreover, multicellular glandular trichomes, unicellular non-glandular trichomes and cystolith trichomes were observed in \textit{M. alba} and \textit{M. nigra}.\textsuperscript{22} In the present study, unicellular non-glandular trichomes of various sizes were found on both leaf surfaces of the studied taxa, but their densities were variable. Glandular trichomes with a unicellular stalk and multicellular head were detected in \textit{M. alba}, \textit{M. nigra}, and glandular trichomes with a unicellular stalk and head were observed in \textit{M. rubra}. We also found peltate glands on the upper surfaces of all studied taxa, as in previous studies.\textsuperscript{22,26,43}

In the Moraceae family, calcium oxalate and carbonate crystals are mostly present in the leaves.\textsuperscript{36,25,33,44,45} Regarding calcium oxalate, two types of crystals (druse crystals in the cells of the mesophyll and bundle sheaths, prismatic crystals only in the cells of the bundle sheath) are located in the leaves of the Moraceae.\textsuperscript{44} The most often seen calcium carbonate crystal type cystolith (a calcified body) are located in several families, such as Urticaceae, Ulmaceae, Moraceae, Cucurbitaceae, and Acanthaceae.\textsuperscript{11,18,46} Many species of the Moraceae are recognized by the presence of cystolith. Cystolith is deposited in a specialized cell called a lithocyst, which is known as an excretory idioblast.\textsuperscript{18,44,46-48} Lithocysts are very common in the Moraceae family and were observed in many anatomical studies on Moraceae. \textit{Ficus} L. species mostly have lithocysts; moreover, they were reported on \textit{Morus} leaves.\textsuperscript{33,37,44,45} According to Esau\textsuperscript{19} the presence and location of crystals may be distinctive and useful in taxonomic classification. In our study, while many druse crystals were found in the mesophyll and also in the midrib region, prismatic crystals were only found in the midrib. Furthermore, lithocysts were noticed only on the upper surface of the leaves of all investigated taxa. In contrast to our study, lithocysts were not observed in some studies on \textit{Morus} taxa.\textsuperscript{22,24,26}

As seen in transverse sections, spongy and palisade parenchyma cells can be distinguished easily from each other in the mesophyll. However, palisade parenchyma layers and their ratio of occupation vary. The leaves of the studied taxa are dorsiventral. The dorsiventral leaf is characteristic of some members of the Moraceae family and is therefore not useful for species identification.\textsuperscript{18,25} Besides, in some works on the Moraceae family, dorsiventral and isobilateral leaves were reported.\textsuperscript{33,37,50-52} In this study, collateral vascular bundles were seen in the midrib region. The differences were determined concerning collenchymatic elements located with various layers between epidermal and parenchyma cells on the midrib of the three taxa.
CONCLUSION

Some differences were determined in the morphological and anatomical properties of all studied taxa. It is obvious that certain characteristics, such as the size, shape, and indumentum of leaves, are helpful in the recognition of taxa. Furthermore, some anatomical characteristics of the leaves were found to be of diagnostic importance, such as the ratio of the density of the palisade parenchyma and collenchyma layers in the midrib region, type, and density of trichomes, length and width of stomata, and SI. All of these characteristics are environmentally influenced, and future studies analyzing plants from several localities are needed; nevertheless, they can be very useful in the delimitation of species.

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