Morphology and microchemical evaluation of *Morus alba* and *M. mesozygia* from Nigeria

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Abstract. The species of genus *Morus* (*Moraceae*) are very useful medicinal plants with known pharmacological effect in the treatment of many diseases and medical conditions. Their ethno-pharmacological potential could be linked to the folklore. This study was carried out in order to compare foliar micromorphology structures and microchemical properties of *Morus alba* and *Morus mesozygia*. Fresh leaf samples of the two plant specimens were collected from the Herbal Garden of Forest Research Institute of Nigeria and prepared for foliar epidermal studies and petiolar transverse section research. Microchemical tests were carried out on dried and ground samples of the plant specimens. The microchemical properties of the two plant specimens were found to be similar, as they possess the same classes of chemicals in their leaves. *M. alba* has shown several unicellular trichomes and simple glandular trichomes, as well as non-glandular straight and non-gandular hooked trichomes; *M. mesozygia* has revealed glandular and non-glandular straight trichomes. The transverse section of *M. alba* had a flattened and shorter adaxial surface, while *M. mesozygia* showed a longer adaxial surface. This evidence is a very useful tool in distinguishing between the various species. Comparative data of the two species were characterized for the first time with distinct anatomical features.

Key words: *Morus alba, Morus mesozygia*, microchemical, microscopy, micromorphology

Introduction

The genus *Morus* L. belongs to the family *Moraceae*, which also houses the genus *Ficus*. The genus *Morus*, popularly known as Mulberry, is composed of approximately 10–16 species distributed across Asia, Africa and North, Central and South America (Nepal & Wichern 2013; Klimko 2016). The most prominent of these species is the White Mulberry (*Morus alba*).

The White Mulberry is native to Southeast Asia, but its cultivation has spread ever since to tropical, subtropical and temperate regions of the world, owing to its varied uses (Zhekum & Gilbert 2003). *Morus alba* has been used in sericulture for thousands of years, mainly because it is known to enhance the growth and development of silkworm caterpillars and thus enhances silk production. It has also been used as a fodder crop due to its high protein content and relatively safe consumption (Srivastava & al. 2006).

The leaves of *Morus alba* have been used in herbal remedies since ancient times and are described in one the earliest Chinese pharmacopoeias (*Divine Husbandman's Materia*) (Bensky & Gamble 1993). It has been used in the treatment of hyperglycemia, inflammation, cough, hypertension, cancer, and fever. Owing to its good therapeutic effect and low toxicity, *M. alba* has been extensively used in conventional Chinese medicine (Yang & al. 2010; Chan & al. 2012; Marx & al. 2016). *M. alba* is also widely studied and has been reported to have neuroprotective, antihyperlipidemic, skin tonic, anti-hyperglyce-

mic, antibacterial, antihypertensive, and antioxidant effect (Ramesh & al. 2014; Rao & al. 2012).

Another important species of the *Morus* genus is the African Mulberry (*Morus mesozygia*) known as Ewe Aye in Yoruba (Nigeria) (Gbile, 1984). It is an endemic species that grows in tropical Africa, where its leaves and fruits are known as a good food source for monkeys and chimpazees in the forests (Fozing & al. 2011). Ethnomedicinal use of *Morus mesozygia* includes the treatment of inflammation, arthritis, stomach disorders and ulcers; pain, and malaria (Baldé & al. 2015; Ntie-Kang & al. 2014).

In this study, foliar anatomy and leaf-powder micromorphological characters of the two species will be shown, in order to find diagnostic characters useful in differentiating the species.

Material and methods

Plant material collection

Fresh mature leaves from the shoots of *Morus alba* and *Morus mesozygia* were collected from the Herbal Garden of the Forestry Research Institute of Nigeria, Ibadan, between February and March, 2019. The specimens were identified and voucher specimens prepared and deposited at the Forest Herbarium Ibadan (FHI), under FHI Nos. 701215 and 701277, respectively.

Light microscopy

Epidermal section

Fragments of 1–5 cm^2 of the leaves of each specimen were cut and soaked in concentrated nitric acid (HNO_3) in tightly covered Petri dishes for about two to four hours, in order to macerate the mesophyll. Tissue disintegration was indicated by bubbles and the epidermises were transferred into clean Petri dishes and adequately rinsed with distilled water, before the abaxial and adaxial layers were separated with forceps. Tissue debris was carefully cleared off the epidermises with a fine Carmel hairbrush, and the isolated epidermal layers were adequately rinsed in water. The epidermises were then transferred into another Petri dish with 50 % ethanol for 1–2 minutes, thereby allowing the cells to harden. Afterwards, the tissues were transferred onto a clear glass microscopic slide, stained with Safranin O for five minutes, and then rinsed again in distilled water to remove the excess staining. They were mounted in 25 % glycerol on a microscopic slide, covered with coverslips and the edges of the coverslip were sealed with nail varnish to prevent dehydration. Five slides were prepared for each epidermis of the two species.

Transverse section

The petioles (tranverse section) of the two species were cut into 10µ sections with a sledge microtome and preserved in 50 % ethanol. They were stained in aqueous solution of Safranin O for about 5 min and rinsed twice in distilled water to remove the excess stain. Thereafter, they were mounted in 15 % glycerol onto glass-microscopic slides and covered with coverslips. Nine sections from the petiole and lamina in the middle parts were taken, with three sections per slide for both plant specimens.

All slides were labelled appropriately and examined under Olympus light microscope at magnification ×10 and ×40 . Photomicrographic images of each specimen were taken with a 14 megapixels Amscope digital camera mounted on Olympus photomicroscope at the Biomedicine Research Centre, Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. Microscope observations and measurements were made with Amscope microscope software Version 3.7.7149 2016.

For each micromorphological character, measurements were randomly taken from all slides prepared for each specimen. The mean value and standard error for all microscopic parameters were also calculated on the basis of occurrence of each examined character in a total of 20 fields of view, as mentioned above.

Powder microscopy and microchemical tests

The plant specimens were air-dried for five days, oven-dried for 24 hours and pulverized to powder in a hammer mill grinder. Pinch of the powder samples was placed on a grease-free microscopic slide, along with a drop of glycerin and water (1:1). It was covered then with a clean coverslip and observed under the compound microscope at 10×. The powder samples were also mounted and stained with phloroglucinol and concentrated hydrochloric acid, diluted iodine solution, ruthenium red, Sudan red and diluted hydrochloric acid.

Results

The characteristics of leaf epidermis of *Morus alba* and *Morus mesozygia* are listed in Table 1 and 2. In both plants, adaxial epidermal cells were irregularly rectangular to polygonal and had straight anticlinal walls. *M. mesozygia* had thicker anticlinal walls, when compared

Table 1. Qualitative microscopic epidermal leaf characteristics of species.

	Morus alba		*Morus mesozygia*	
Characters	**Abaxial**	**Adaxial**	**Abaxial**	**Adaxial**
Cell shape	Irregular Rectangular Polygonal	Irregular Rectangular Polygonal	Irregular Rectangular Polygonal	Irregular Rectangular Polygonal
Anticlinal wall	Straight	Straight	Straight	Straight
Stomata Type	Anomocytic	Absent	Anomocytic	Absent
Idioblast	Absent	Round or Oval	Absent	Round or Oval
Trichomes		Glandular Non-glandular Straight Non-glandular Hooked		Glandular Non-glandular Straight

Table 2. Quantitative microscopic epidermal leaf characteristics of species.

	Morus alba		*Morus mesozygia*	
Characters	**Abaxial**	**Adaxial**	**Abaxial**	**Adaxial**
Cell length (µm)	26.85–16.17 (23.66±1.93)	42.60–22.33 (31.13±2.01)	28.66–25.90 (27.79±0.49)	32.39–23.77 (28.91±0.96)
Cell width (µm)	11.52–6.82 (9.05±0.82)	26.54–17.76 (22.55±0.86)	25.12–14.10 (21.18±1.92)	32.85–20.44 (24.24±1.30)
Stomata length (µm)	15.22–11.33 (13.15±0.46)	–	23.24–11.07 (15.36±1.13)	–
Stomata width (µm)	10.63–6.82 (8.55±0.39)	–	13.74–8.46 (11.42±0.55)	–
Idioblast length(µm)	–	61.88–34.23 (45.41±3.00)	–	59.00–47.25 (54.23±2.49)
Idioblast width(µm)	–	42.22–23.5 (32.37±1.73)	–	56.08–35.83 (45.77±4.01)

Max–min
(Mean±standard Error)

to *M. alba.* Quantitative data indicated that epidermal cells on the adaxial and abaxial surfaces of *M. alba* averaged 31.13±2.01 µm (length) by 22.55±0.86 µm (width) and 23.66±1.93 µm (length) by 9.05±0.82µm (width), respectively. While for *M. mesozygia*, the average epidermal cells for adaxial and abaxial surfaces were 28.91±0.96 µm (length) by 24.24±1.30 µm (width) and 27.79±0.49 µm (length) by 21.18±1.92 µm (width), respectively. Stomata were found only on the abaxial surfaces of the leaf in both plant species, with anomocytic arrangement of 5–7 cells surrounding the guard cell.This implies that the plant species are hypostomatic, with stomata on the abaxial surface of their leaves, as seen in Figs 1 and 2. The average stomata measured 13.15±0.46 µm (length) by 8.55±0.39 µm (width) for *M. alba,* and 15.36±1.13 µm (length) by 11.42±0.55µm (width) for *M. mesozygia.*

Idioblasts were found only on the adaxial surfaces of both species, while trichomes were found on both surfaces, although there were more trichomes on the adaxial than on the abaxial surface in both species. *M. alba* had unicellular trichomes: simple sessile glandular trichomes, non–glandular straight and non-gandular hooked trichomes. *M. mesozygia* had unicellular sessile glandular and straight non-glandular trichomes only (Fig. 5).

A transverse section (Figs 3 and 4) of the leaves revealed an open collateral arrangement of the vascular bundle in both species. The vascular system occupied half of the lamina in the cross-section in *M. mesozygia* but less than that in *M. alba.* Cuticles of *M. alba* proved thicker than in *M. mesozygia*. Collenchyma was absent towards the abaxial surface but appeared close to the adaxial surface in both plants, with 3–4 cell layers in *M. mesozygia* and 5–6 cell layers in *M. alba*. The vascular

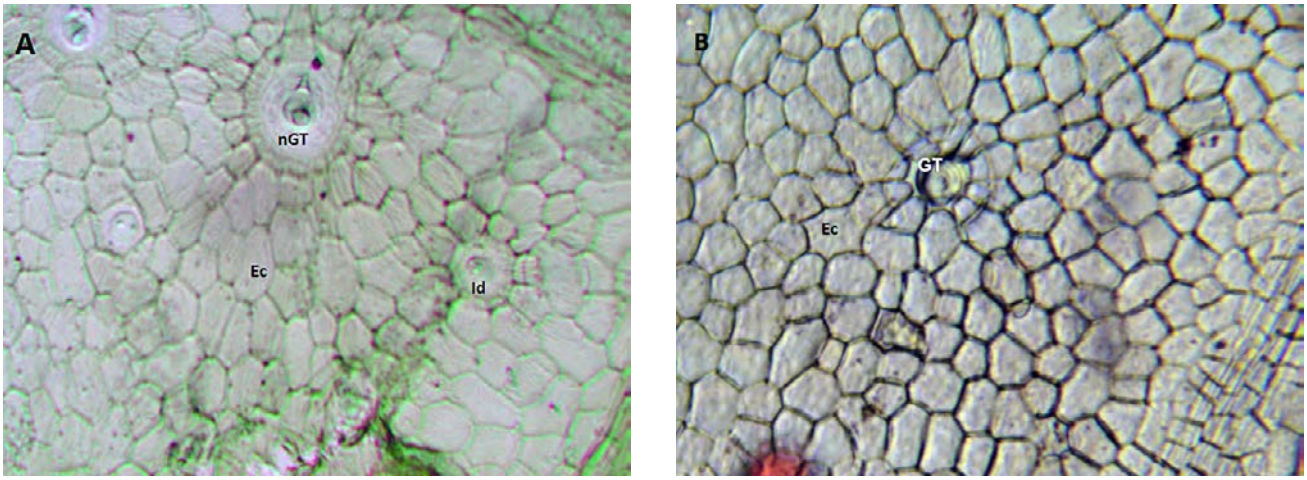

Fig. 1. Adaxial Epidermal Section of *Morus alba* **(A)** and *Morus mesozygia* **(B)**. (**Ec**= Epidermal cells, **nGT**= Non Glandular Trichomes, **Id**= Ideoblast, **GT=** Glandular trichome

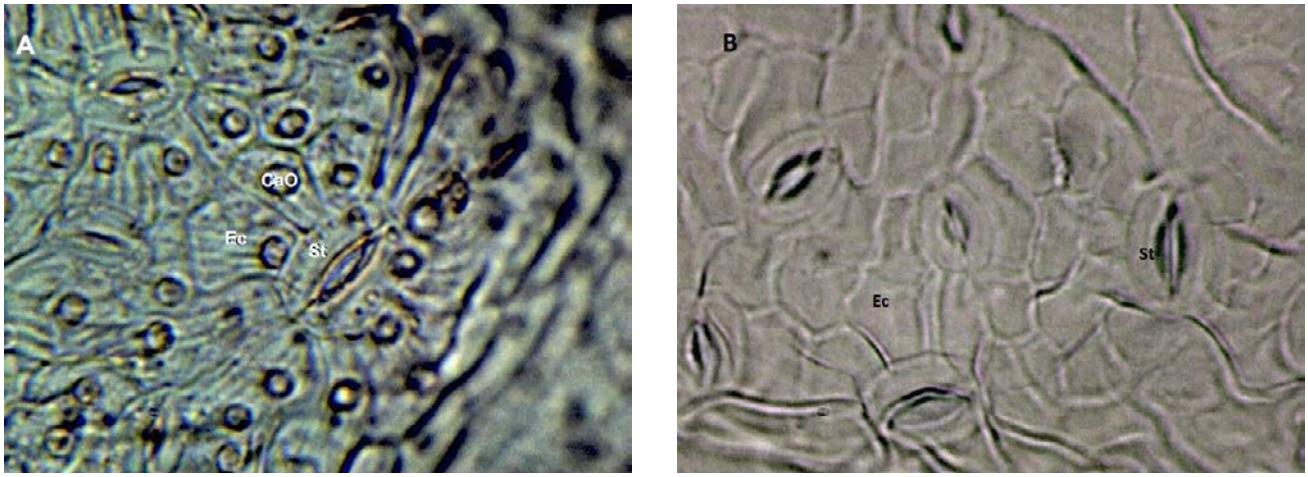

Fig. 2. Abaxial Epidermal Section of *Morus alba* **(A)** and *Morus mesozygia* **(B)**. (**Ec**= Epidermal cells, **St**= Stomata, **CaO**= Calcium Oxalate Crystals)

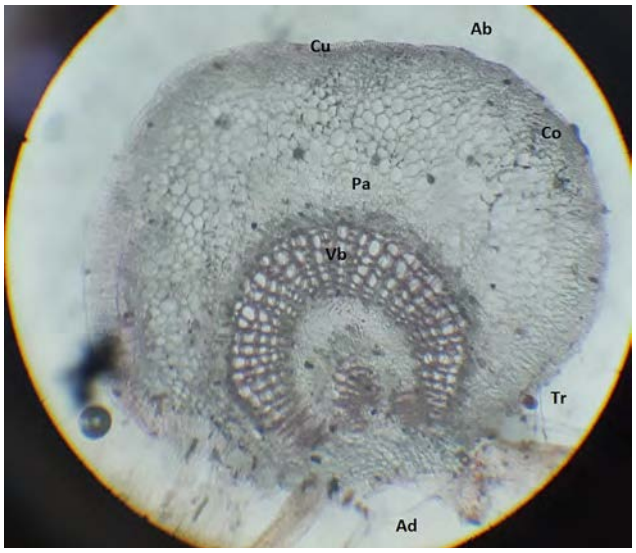

Fig. 3. Transverse section of *Morus alba*. (**Cu**= Cuticle, **Vb**= Vascular Bundle, **Co**= Collenchyma, **Pa**= Parenchyma, **Tr**= Trichomes, **Ad**= Adaxial layer, **Ab**= Abaxial Layer)

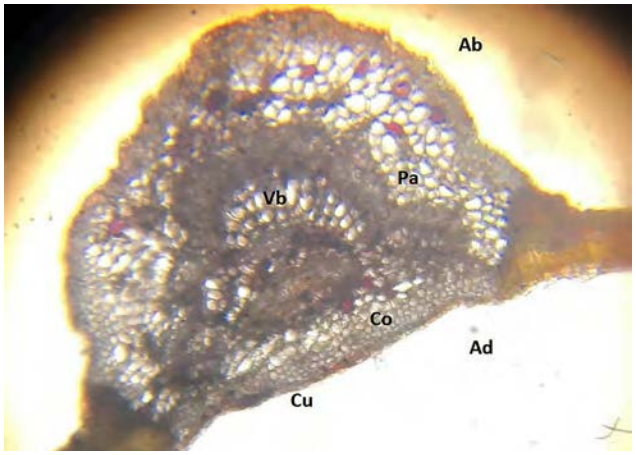

Fig. 4. Transverse section of *Morus mesozygia*. (**Cu=** Cuticle, **Vb**= Vascular Bundle, **Co=** Collenchyma, **Pa=** Parenchyma, **Tr=** Trichomes, **Ad=** Adaxial layer, **Ab=** Abaxial Layer)

system resembled abaxial arc in both species: in *M. alba* the arc was more enclosed, tending towards a circular structure, whereas the opening of *M. mesozygia* was broader. This clearly distinguished the transverse sections of the species: *M .alba* with a flattened and shorter adaxial surface and *M. mesozygia* with a longer adaxial surface. Vascular bundles were centrally placed, with a 4–6 celled xylem for *M. alba* and a 2-4 celled xylem for *M. mesozygia,* forming a convex arc for the phloem.

Powder microscopy

Calcium oxalate crystals in *M. mesozygia* were observed as rectangular to polygonal shapes on the epidermal cells in the veins (Figs 5 and 6). In *M. alba*, circular and polygonal calcium oxalate crystals were found in druses, mostly inside the epidermal cells on the abaxial layers. Fragments of straight non-glandular trichomes were observed in both plant specimens but hooked non-glandular trichomes were found only in *M. alba.*

The result of the chemomicroscopy tests of the powdered samples showed the presence of lignified cells, fats (cuticles) and calcium oxalate crystals in both species, as well as absence of starch and mucilage (Table 3).

Table 3. Microchemical tests.

Test	Reagents	*M. alba*	*M. mesozygia*
Lignified cells	Phloroglucinol + Conc. HCl	+ve	+ve
Mucilage	Ruthenium Red	–ve	–ve
Fat (cuticle)	Sudan Red III	+ve	+ve
Starch	Dilute Iodine Solution	–ve	–ve
Calcium Oxalate	Dilute HCl	+ve	+ve

+ve indicates a positive test
–ve indicates a negative test

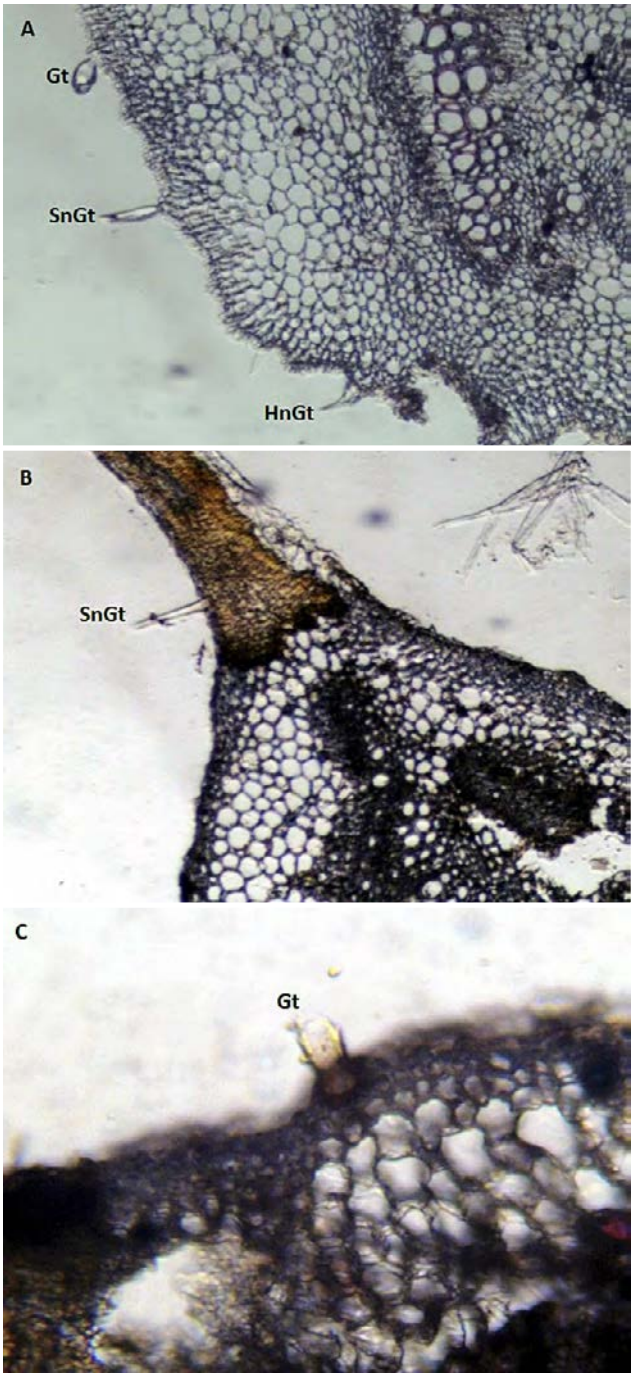

Fig. 5. Types of trichomes found in *M. alba* (**A**) and *M. mesozygia* (**B**) (**C**). (**Gt**= Glandular Trichome, **SnGt**= Straight non glandular Trichome, **HnGt**= Hook non glandular Trichome)

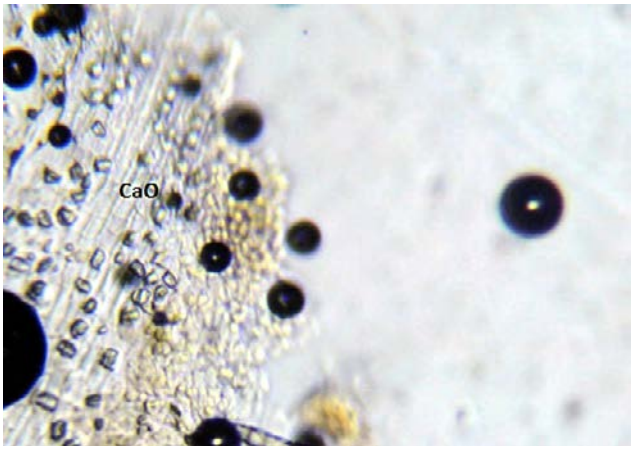

Fig. 6. Powder fragment of *Morus mesozygia*. (**CaO**= Calcium oxalate Crystals)

Discussion

Micromorphogical data has been useful in the taxonomic classification of plants because it reveals the inherent variations within the species, genera or families. These data has been used frequently in phamacognostic evaluation and quality control of herbal medicines (Gohil & *al.*, 2015; GHP 2003). Leaf epidermal and transverse sections provide ample variable characteristics, such as stomata types, trichomes, presence of crystals, vascular bundles etc., which can be used to distinguish plants (Chandaz 2014; Evans 2009).

Some of the clearly distinguishing features of *M. alba* and *M. mesozygia* are the differences in epidermal cell and stomata size, in *M. alba* with comparatively larger epidermal cells and smaller stomata size. Furthermore, the presence of hooked trichomes in *M. alba* is a major feature that distinguishes it from *M. mesozygia,* in which no hooked trichomes have been observed. Glandular trichomes present in both species mostly indicate a role in secretion of some specific phytochemicals by the species that could serve as a natural defense mechanism against herbivores, insects and microbes (Joris & *al.*, 2012).

Apart from these features, most other features in both species are similar as in other members of the *Moraceae* family, showing their close relationship (Truchan 2015). Both species are hypostomatic, with stomata only on their abaxial surface. Presence of ideoblasts discovered in this study on the adaxial surface in both species is a common feature for genus *Morus* reported by Klimko in 2016. The petiolar transverse sections of the species slightly differ in shape in *M. alba* by a shorter and flattened adaxial portion leading to a more circular abaxial portion, when compared to *M. mesozygia,* whose adaxial portion is longer and abaxial portion resembles more a semi-circular arc.

The presence of lignified tissues in both powdered samples evidences the supportive and protective roles lignin plays in many plant species. Calcium oxalate crystals present in both species differ in *M. mesozygia*

by tending towards a rectangular to polygonal shape, while those found in *M. alba* resemble circular druses. The presence of these crystals and fatty cuticles is indicative for the other species of the genus *Morus,* as well as for some sections of the *Moracae* family (Klimko 2016).

Conclusion

The results of this study provide for the first time comparative data on the micromorphological and microchemical characteristics of *M. alba* and *M. mesozygia.* Some characters stand out as differentiating factors for both species. Most notable are the absence of hooked non-glandular trichomes in *M. mesozygia*, differences in size of the epidermal cells, and the shape and location of calcium oxalate crystals. Most of the other observed characteristics are similar and in line with those reported in several studies into other plants of the genus (Truchan 2015; Klimko 2016). In addition to powder microscopy and microchemical tests, the reported micromorphological characters provide some parameters for standardization, considering the fact that these plants are very important ethno-medicinally and contain many pharmacological potentialities that have to be unraveled yet. This study would also be useful for correct taxonomic identification and authentication of the plant species.

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