

COMPARATIVE MORPHOLOGY AND ANATOMY OF THE LEAF
AND STEM OF SPECIES OF *ZANTHOXYLUM* (RUTACEAE)
FROM CENTRAL ARGENTINA

MORFOLOGÍA Y ANATOMÍA COMPARADA DE LA HOJA Y EL TALLO
DE LAS ESPECIES DE *ZANTHOXYLUM* (RUTACEAE)
DEL CENTRO DE ARGENTINA

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ABSTRACT

Zanthoxylum is one of the major genera of Rutaceae with at least 200 species in tropical and temperate regions worldwide. In Argentina is represented by 10 species, two of them inhabiting in the central region, the native *Zanthoxylum coco* and the naturalised *Zanthoxylum armatum* var. *armatum*. These species are found in xerophytic deciduous forests of Chaco biogeographic province. Besides the floristic and ethnobotanical importance of these species, their leaves and stems have been scarcely studied. The objective of this contribution was to present a comparative morphological and anatomical study of the leaves and young stems of the species of *Zanthoxylum* from central Argentina. The analysis was done using herbaria vouchers, freshly collected and fixed material. The species share morphological features, as armed stems, leaves alternate, odd-pinnately, 3- to many foliolate. The main differences are that *Z. armatum* is an evergreen shrub with axillar inflorescences

whereas *Z. coco* is usually a deciduous tree with terminal inflorescences. Both species also share the presence of secretory cavities, glandular trichomes and hypostomatic leaves. However each species presents particularities of the leaves structure that could be important and related to the environment that the plants develop.

Key words: *Zanthoxylum armatum*, *Zanthoxylum coco*, xeric habitats, flora.

RESUMEN

Zanthoxylum es uno de los principales géneros de Rutaceae con al menos 200 especies de las regiones tropicales y templadas de todo el mundo. En Argentina está representado por 10 especies, dos de ellas habitan en la región central, *Zanthoxylum coco*, nativo, y el naturalizado, *Zanthoxylum armatum* var. *armatum*. Estas especies se encuentran en los bosques caducifolios xerofíticos de la provincia biogeográfica del Chaco. A pesar de la importancia flo-

rística y etnobotánica de estas especies, sus hojas y tallos han sido escasamente estudiados. El objetivo de esta contribución es presentar un estudio morfológico y anatómico comparativo de las hojas y tallos jóvenes de las especies de *Zanthoxylum* del centro de Argentina. El análisis se realizó utilizando ejemplares de herbario, material fijado y fresco. Las especies comparten rasgos morfológicos, como tallos armados, hojas alternas, imparipinnadas, 3- multifolioladas. Las principales diferencias son que *Z. armatum* es un arbusto de hojas perennes con inflorescencias axilares mientras que *Z. coco* suele ser un árbol de hojas caducas con inflorescencias terminales. Ambas especies también comparten la presencia de cavidades secretoras, tricomas glandulares y hojas hipostomáticas. Sin embargo, cada especie presenta particularidades de la estructura de las hojas que pueden ser importantes y relacionadas al ambiente en el que las plantas se desarrollan.

Palabras clave: *Zanthoxylum armatum*, *Zanthoxylum coco*, ambientes xéricos, flora.

INTRODUCTION

The family Rutaceae Juss. is placed in order Sapindales primarily because of the pinnately compound leaves, absence of resin ducts in the bark, wood rays, leaf veins, the presence of triterpenoid compounds and flowers with a distinct nectar disk (Cronquist, 1992; AGP III, 2003; Judd *et al.*, 2008) and includes species of great economic importance as ornamental plants, medicinal or culinary uses or perfume industry such as the orange, lemon, lime, kumquat, mandarine and grapefruit. The Rutaceae are represented in Argentina by seven genera and approximately 18 species (Seo and Xifreda, 2008).

They are shrubs, trees, or sometimes herbs, sometimes scrambling or scandent, sometimes armed, with aromatic volatile oils contained in glands visible at surface of, at least leaves, young branchlets, inflorescences, flower parts, fruit, or cotyledons in seed. The genus *Zanthoxylum* L. is one of the major genera of Rutaceae with at least 200 species in tropical (mainly) and temperate regions of the world (Figueiredo Melo & Zickel, 2004; Zhang & Hartley, 2008). It includes shrubs sometimes scrambling, trees, or woody climbers, evergreen or deciduous, usually armed. The leaves are alternate, odd-pinnately 3- to many foliolate or sometimes digitately 3-foliolate. *Zanthoxylum* is represented in Argentina by 10 species, two of them inhabiting in central region of the country, *Zanthoxylum coco* Gillies ex Hook. f. & Arn. and *Zanthoxylum armatum* DC var. *armatum*. These taxa are the only species of *Zanthoxylum* found in Pampean hills of central Argentina, which belong to the biogeographic province of Chaco (Morrone, 2001; 2004). This province is characterized by xerophytic deciduous forests of *Vachellia caven* (Molina) Seigler & Ebinger, *Aspidosperma quebracho-blanco* Schlttdl., *Celtis ehrebergiana* (Klotzsch) Liebm., *Lithraea molleoides* (Vell.) Engl. and *Zanthoxylum coco* as predominant species of trees. Although these species are studied in floristic, taxonomic and ethnobotanic treatments, there are very scarce morphological and anatomical studies of vegetative organs of these species.

Anatomy describes the internal structure of plants and is considered as a source of correct identification of taxa. Anatomy centres on the spatial arrangement of the dermal, ground, and vascular tissue systems (Nancy & Dengler, 2002). Similarly foliar

epidermal microscopic features like shape of epidermal cell, type of stomata, presence or absence of pubescence and cell wall thickness are also considered as useful tools for correct taxa identification and their phylogenetic relationship with other taxa (Stace, 1965; Babalola & Victoria, 2009).

Although *Zanthoxylum coco* is one of the most common species of trees in forests in central Argentina Hills and it is used as medicinal plant (Battista *et al.*, 2007), their leaves and stems have not previously been studied. The shrub *Zanthoxylum armatum* is an important medicinal plant extensively used, in the countries which is native, for curing *Pneumonia*, tick infestation and gum diseases (Iqbal & Hamayun, 2005), fruit is used for toothache, dyspepsia, and seeds are used as condiment and flavoring agent (Arshad & Ahmad, 2005; Abbasi *et al.*, 2010). Recently the leaves and fruits were investigated for various pharmacological activities and has showed good results including cytotoxic, phytotoxic, antipyretic activities and rationalise its local uses (Sukathan *et al.*, 2009; Barkatullah *et al.*, 2011) and in Argentina was found as naturalized species (Arana & Oggero, 2009). Keeping in view the significance of morphological and anatomical studies for taxonomy and ecology, a comparative morphological and anatomical study of the leaves and young stems of the species of *Zanthoxylum* from central Argentina is carried out.

MATERIAL AND METHODS

Plant material

The collections of the botanical material and the field observations were carried out in Sierra de Comechingones, located in the

southwestern part of Cordoba province, Argentina (fig. 1).

The comparative morphological description of the leaves and stem was carried out using fresh material and herbaria specimens and it is based in Escalante (1961) for *Zanthoxylum coco* and Arana & Oggero (2009) for *Zanthoxylum armatum*. The herbaria consulted were CORD, LIL, LP, SI, RCVC and RIOC, and digital images of the types (provided by G and K) were also seen. The selected samples of the specimens collected were deposited in the Herbarium of Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto (RCVC) and registered as follows:

Zanthoxylum armatum:

ARGENTINA. Córdoba. Depto. Calamuchita, Embalse Cerro Pelado, A. J. Oggero & M.D. Arana s.n. (RCVC); Villa Amancay, 14-III- 2009, M. D. Arana & A. J. Oggero s.n. (RCVC); Depto. Río Cuarto, Alpa Corral, 30- III-2000, C. A. Bianco, s.n. (RCVC 3625).

Zanthoxylum coco:

ARGENTINA. Córdoba. Depto. Calamuchita, Embalse Cerro Pelado, 14-III- 2009, A.J. Oggero & M.D. Arana s.n. (RCVC); Depto. Río Cuarto, Sierra de Los Cóndores, 10- X- 1997, M. Ceballos, s.n. (RCVC 3948).

Morphological studies

The analysis of the stem and leaf structure was done using freshly collected material or fixed material. The leaves located in the nodes of the middle part of the newest branchlets (there is one leaf per node along the stem) were used to carry out the

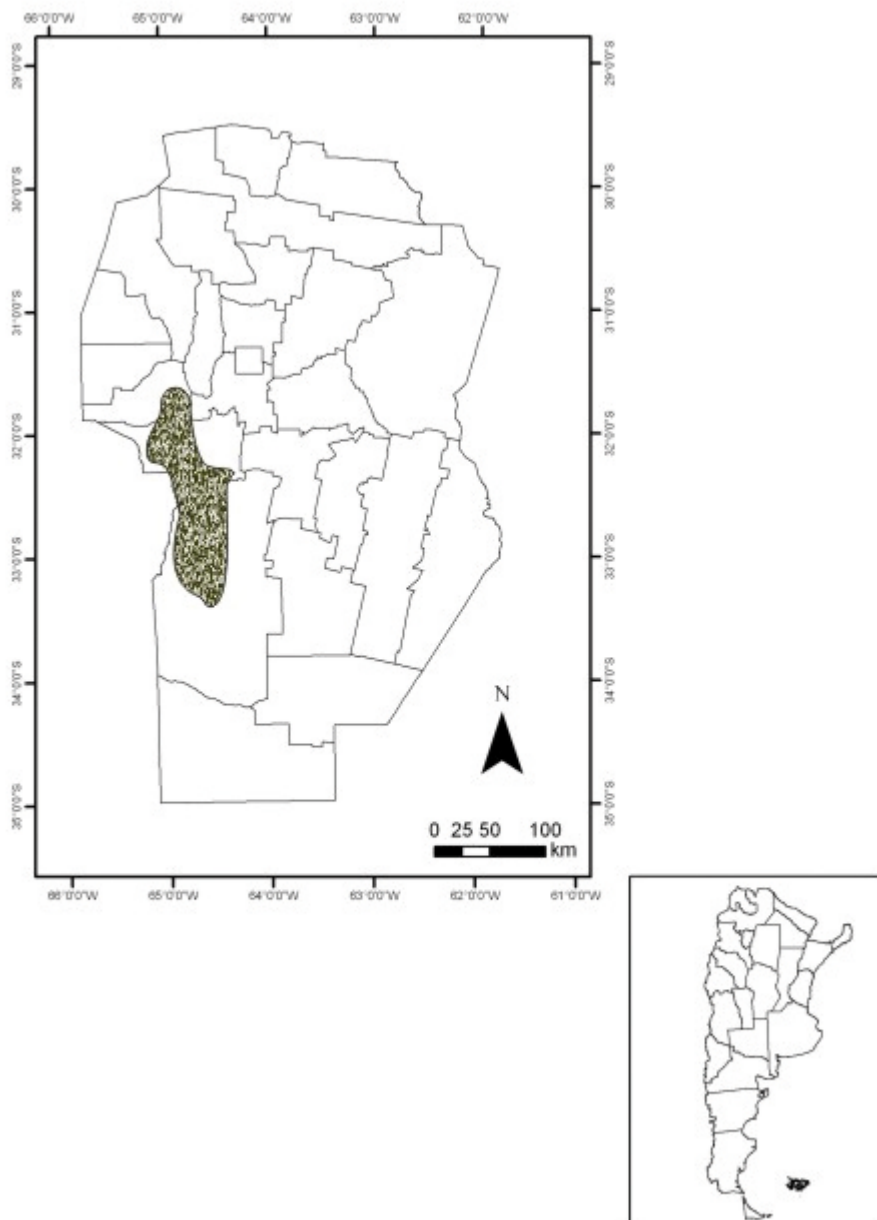


Fig.1. Location of Sierra de Comechingones (central Argentina) where the study was carried out.

anatomical study. The samples were taken from the central zone of the middle leaflet and the rachis. To anatomical studies of stem, the internodes (a portion of a plant stem between nodes) of the middle region of the branchlets were collected.

The samples were placed in FAA (95% ethanol: glacial acetic acid: 37-40% formaldehyde: water; 50:5:10:35, v/v). The dehydration of samples was done according to the procedures outlined in Johansen (1940) using graduated solutions of ethanol and xylene. Fully infiltrated tissues were embedded in Histowax (highly purified paraffin wax blended with polymer additives). A series of transverse sections 12 µm thick were obtained from the sample blocks using a Minot rotary microtome. The sections were triple-stained with hematoxylin, safranin O and fast green FCF as described by Johansen (1940). To verify the presence of starch, semi-permanent slides with Iodine dissolved in an aqueous solution of Potassium iodide (Lugol's Iodine solution) colored were made.

Epidermal studies were carried out using leaflet samples fixed in FAA which were submerged in a solution 40% of sodium hypochlorite and heated to epidermal separation. The epidermal samples were washed with water and safranin coloured and the semipermanent slides for microscope using water-glycerine (1:1) solution were made, according to the usual techniques.

The stomatal index (SI) of each taxon was calculated from five fields in the middle region on lower surface of the blade of six leaves taken from different plants, using the formula proposed by Cutter (1969).

The stomatal density, trichomes and (just in case of *Zanthoxylum coco*) glands were counted using ten microscope fields (0.25 mm²) on each epidermis of six leaflets.

A standard Zeiss Model 16 microscope was used to assess the histological preparations and photomicrographs were taken with a *Zeiss Axiophot* microscope with an equipment of image capture and digitalization AxioVision 4.3, with camera *AxioCam HRc*.

RESULTS

Zanthoxylum armatum var. *armatum*

Young stem and leaves morphology

The plants of *Zanthoxylum armatum* are shrubs to 5 m tall, deciduous, with dark brown cylindrical stems, alternate phyllotaxis and compound, imparipinnate leaves (fig. 2b). Stems, branchlets and leaflet blades abaxially on midvein with dark brown, stout prickles with a broad flattened base. Young branchlets and inflorescence rachises are glabrous or rust-colored pubescent. The leaves are 3-5(7) foliolate with rachis and petioles glabrous or rust-colored pubescent, broadly winged, wings to 6 mm on each side. The leaflet blades are dark green coloured, subsessile, opposite, narrowly elliptic (5-15 × 1-3 cm), base attenuate to broadly cuneate, the terminal leaflet is the largest. The margin is crenate, finely serrulate or entire and revolute when dry, apex acute to acuminate, with the midribs impressed above, elevated beneath. The lateral veins are 6-7 on each side of the midrib with pellucid gland on margins.



Fig. 2. Newest branchlets of *Zanthoxylum* (a) *Z. coco* and (b) *Z. armatum*.

Anatomy of leaves and young stems

The leaflets are dorsiventral. In transverse section the epidermis of adaxial surface present isodiametric cells bigger than the abaxial surface. The stomata are present in abaxial surface and are at the same level of the surrounding cells. The palisade tissue is unilayered, represents the 50% of the mesophyll and it is continuous, even in the midrib region (fig. 3a, b). The secretory cavities are present in the margins of the leaflets only. The vascular bundles are collateral, small and are proximal each other and have bundle sheaths (fig. 3c).

The midrib is surrounded by parenchyma and it is very prominent in the abaxial surface (fig. 3a).

The rachis is winged (fig. 3d). The epidermis of the wings is similar to the leaflets but it is observed a difference in the proportion of the palisade tissue, which represent less than the 50% of the mesophyll (fig. 3d). The central zone of the rachis presents a pith surrounded by xylem and phloem, where secondary growth is observed. The secondary phloem is in contact with chlorenchyma in the abaxial surface, where a layer of collenchyma is also found (fig. 3e).

The epidermis of both, the leaflets and rachis, present peltate trichomes, which are glandular and multicellular. The trichomes are sunken in the epidermis (fig. 3e).

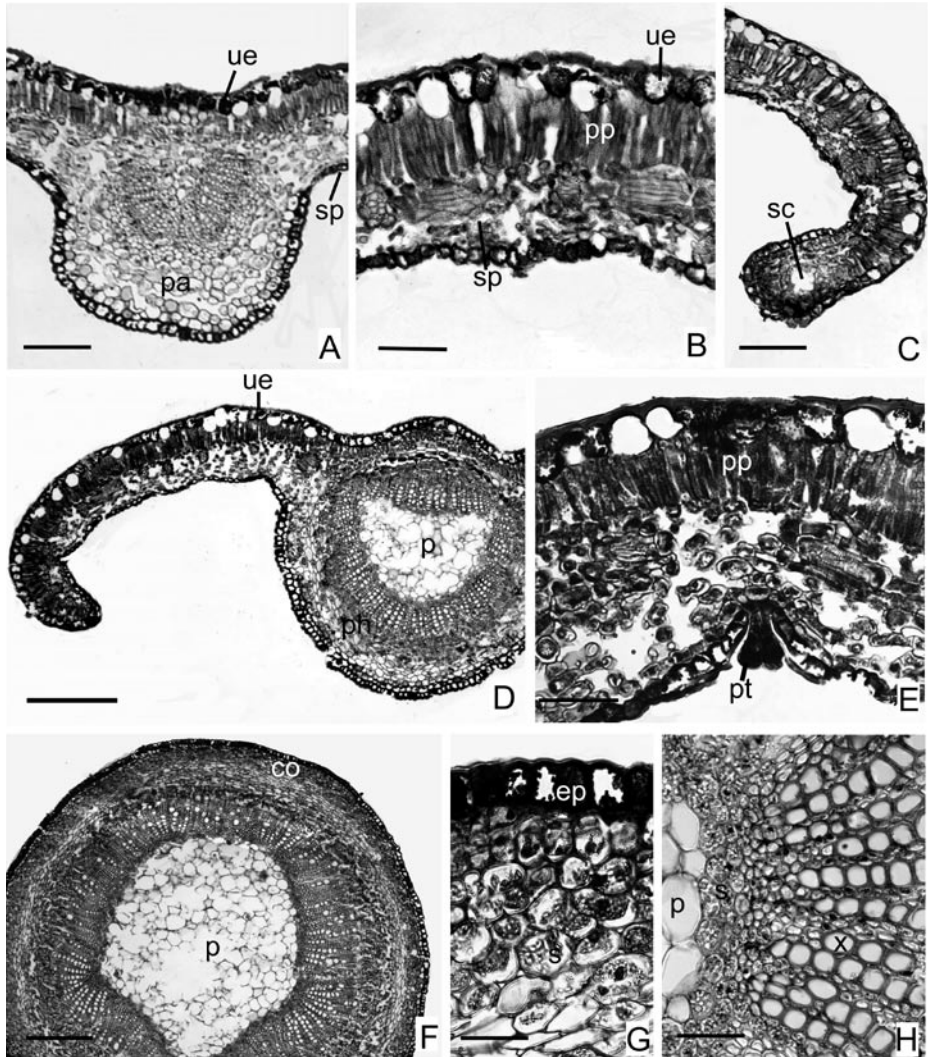


Fig. 3. Micrographs in transverse section of leaves and young stems of *Zanthoxylum armatum* (A) midrib region of leaflet (B) detail of mesophyll (C) margin of the leaflets with secretory cavities, (D) midrib region of rachis, (E) detail of wing of rachis with peltate trichome, (F) young stem (G) and (H) detail the different zone of showing the starch in cells. co, cortex; ep, epidermis; p, pith; pa, parenchyma; ph, phloem; pp, palisade parenchyma; pt, peltate trichome; s starch; sc, secretory cavity; sp, spongy parenchyma; xi xylem; ue, upper epidermis. Scale bars = 50 μm (a), (c), (e); 40 μm (b), (f); 100 μm (d), (h), and 20 μm (g), (i), (j).

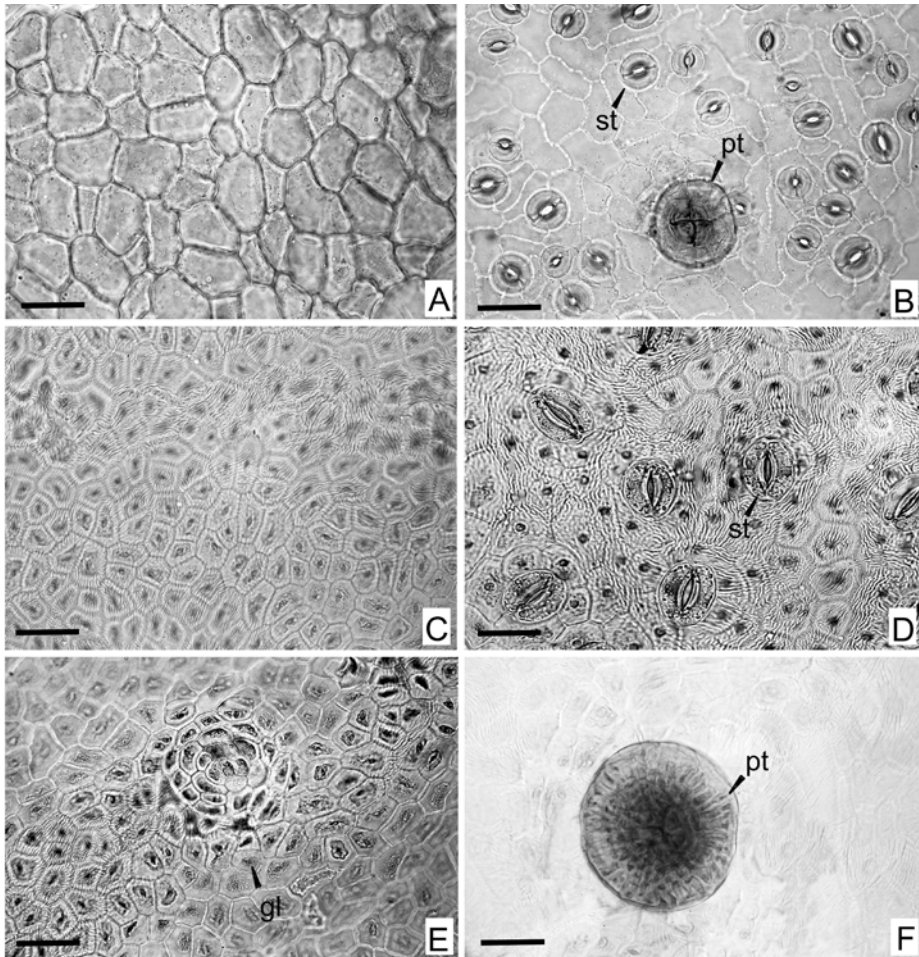


Fig. 4. Micrographs of leaf epidermis of *Zanthoxylum armatum* (A) adaxial epidermis, (B) abaxial epidermis with peltate trichome; and *Zanthoxylum coco* (C) adaxial epidermis, (D) abaxial epidermis, (E) gland on adaxial epidermis, (F) peltate trichome. gl, gland; pt, trichome peltate; st, stoma. Scale bar = 50 μ m.

The epidermis of leaves is unilayered in both, the adaxial and abaxial surfaces and present a slight striated cuticle (fig. 4a, c). The epidermal cells of the adaxial surface have straight walls and are pentagonal or quadrangular of different sizes, while the cells of the abaxial surface are irregular with sinuous anticlinal walls. There are multicellular peltate hairs in both epidermal faces. These trichomes present a multicellular peduncle and a head with four central cells with strong walls and numerous peripheric cells (fig. 4b). The average density of the trichomes was 3 per mm² in abaxial surface and lower than 1 per mm² in adaxial surface. The leaves are hypostomatic, the stomata are anomocytic and present different sizes and a heterogeneous distributional pattern, some of them are so closed that the occlusive cells of the neighbouring stomata are in contact (fig. 4d). The average SI was 22.4 whereas the stomatal density was 252.3 mm².

The young stems present a dermal tissue that consists in a unilayered epidermis and a cortex formed by storage parenchyma subepidermic strata with abundant starch granules (fig. 3g). The central zone of the stem presents a parenchymatous pith is surrounded by parenchyma with abundant starch granules (fig. 3h) and secondary xylem forming a continuous cylinder with rays. A cambial zone separated the secondary xylem from the secondary phloem (fig. 3f).

Zanthoxylum coco

Young stem and leaves morphology

The plant is a tree to 8 m tall, deciduous, with grey armed stems with short grey

stout conic prickles when young and armed branchlets with straight pale brown prickles. The phyllotaxis is alternate with compound, imparipinnate or rarely paripinnate leaves (fig. 2a). Young branchlets and inflorescence rachises are glabrous or minutely pilose, sulcate. The leaves are 3-7 foliolate with rachis and petiole glabrous, without wings. The rachis is sulcate, with two prominent glands in each node. The leaflet blades are membranaceous, glabrous, pale green coloured, with transparent oil glands dispersed on all surface. The leaflets are sessile or pedicellate, opposite, narrowly obovate, base attenuate and obtuse apex, the terminal leaflet is similar to the lateral ones. The margin is crenate or serrulate and not revolute when dry. Rachises and petioles are armed with pale brown, straight or slightly curved prickles.

Anatomy of leaves and young stems

In transverse section of the leaflets blade, the cells of both epidermal faces tissue are similar in form and size and are protected by a thick cuticle. The stomata in abaxial face of the leaflet are at the same level of the surrounding epidermal cells. In the same face of the blade there is an important vascular bundle that shows a secondary growth, whereas to the adaxial face there is one little collateral vascular bundle that shows an inverted position. The vascular tissue is surrounded by parenchyma and it is prominent in both surfaces of the leaflet. The mechanical tissue is absent in this area (fig. 5a). In the mesophyll the vascular bundles are collateral and surrounded by a bundle sheath. The palisade tissue constitutes the major part of the mesophyll and occurred on either side of the spongy mesophyll. The cells of the palisade tissue in the adaxial surface

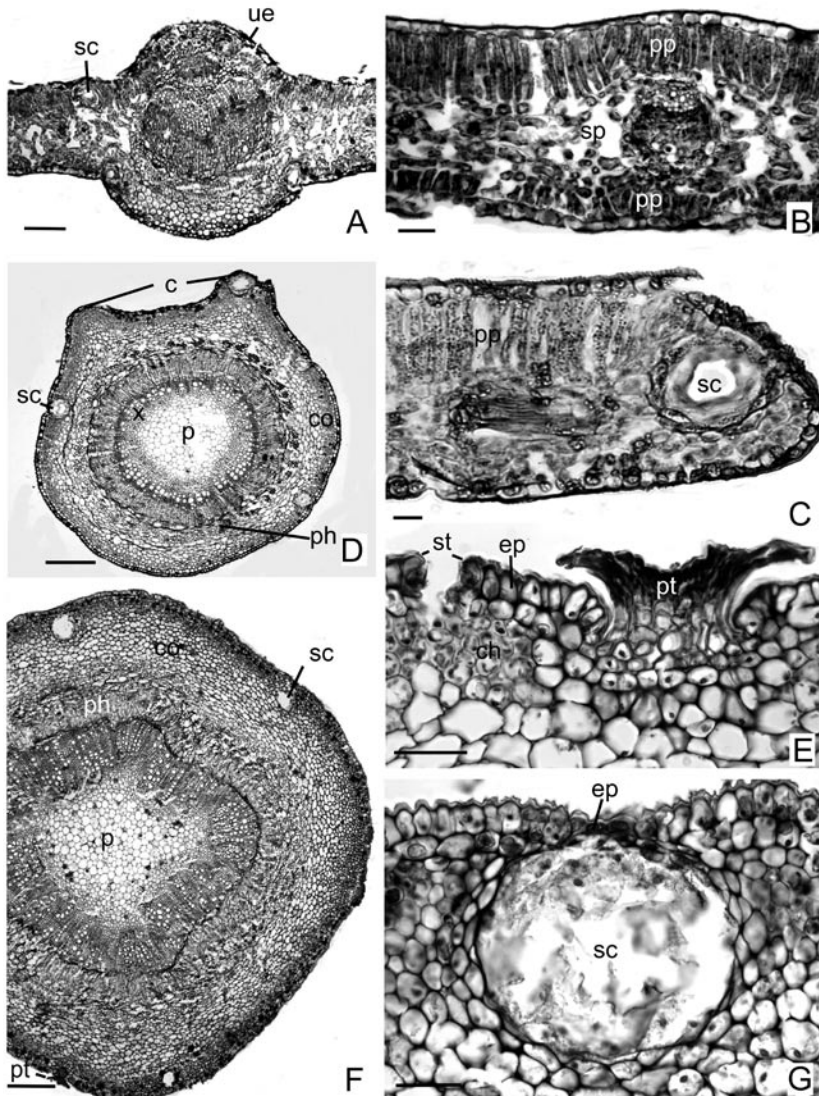


Fig. 5. Micrographs in transverse section of leaves and young stems of *Zanthoxylum coco* (a) midrib region of leaflet (b) detail of mesophyll (c) margin of the leaflets with secretory cavity, (d) rachis, (e) detail of (d) showing peltate trichome, (f) young stem, (g) detail the (f) showing secretory cavity. c, carenae; ch, chlorenchyma; co, cortex; ep, epidermis; p, pith; pa, parenchyma; ph, phloem; pp, palisade parenchyma; pt, peltate trichome; sc, secretory cavity; sp, spongy parenchyma; xi xylem; ue, upper epidermis.

Scale bars = 50 μ m (a), (b), (c), (e) and 100 μ m (d), (f), (g).

are long and narrow, while the cells of the abaxial surface are short and wide (fig. 5b). In certain zones of the mesophyll the palisade parenchyma tends to disappear and is replaced by the spongy one.

The secretory cavities are present in all parts of the leaflets, even in the margins (fig. 5c). These secretory cavities are considered lysigenous because cellular remains in the borders of the cavities are observed. The secretory cavities are also found in all analyzed parts of the plant.

The *Zanthoxylum coco* epidermis of leaves is unilayered in both, the adaxial and abaxial surfaces and the cuticle is striated. The epidermal cells are similar in both surfaces, they are polygonal and present different sizes, with straight or slightly curved anticlinal cell walls (fig. 4d, e). There are notorious multicellular peltate trichomes in both surfaces. These trichomes present a multicellular peduncle and a head with many central and peripheral cells (fig. 4f). Also there are multicellular glands in both epidermis, the average density of these glands was 5 per mm² in the adaxial surface and 3 per mm² in the abaxial surface (fig. 4e). The density of the trichomes was 4.5 per mm² in abaxial surface and 1.5 per mm² in adaxial surface. The leaves are hypostomatic, the stomata in the abaxial surface are separated by similar distances among them but they not showed a specific distributional pattern (fig. 4e). The predominant type of stomata is anomocytic and there are some cyclocytic, both types of stomata are surrounded by 4-6 epidermic cells. The average SI was 8.03, and the stomatal density was 189.5 mm².

The rachis is bicrenated and the carenae are in the adaxial surface. The epidermis is

unilayered with stomata in the same level that the surrounding cells. The epidermis presents multicellular trichomes, which are peltate and glandular, located in cavities of the surface. Under the epidermis there are chlorenchyma interrupted by secretory cavities. There is a continuous cylinder of xylem and phloem surrounding a parenchymatous pith (fig. 5d, e). The young stems (fig. 5f) also present a unilayered epidermis covered by a thick cuticle protecting the parenchymatous cortex, which presents secretory cavities of relatively big size near to epidermis (fig. 5g). The epidermis is interrupted by schlerenchyma, with cells highly lignified, in the areas where the prickles are present. The vascular tissue has the same organization as the described for the rachis.

DISCUSSIONS AND CONCLUSIONS

The studied *Zanthoxylum* species share morphological features, as armed stems, leaves alternate, odd-pinnately, 3- to many foliolate. The main differences are that *Z. armatum* is an evergreen (or sometimes deciduous) shrub with axillar inflorescences whereas *Z. coco* is usually a deciduous tree with terminal inflorescences.

Study of different types of tissues and other microscopic techniques like linear measurements, determination of leaf constants and quantitative microscopy are indispensable in the initial identification of plants materials (Jarald & Jarald, 2007). Some observed features in both species are characteristic of plants that inhabit places with xeric conditions as occurred in central hills of Argentina. Both species share the presence of secretory cavities, glandular trichomes and hypostomatic leaves. However each species presents particularities that could be important and

related to the environment that the plants develop. These features are basically related to the leaves structure, owing to the fact that the leaf is the most sensitive organ and the first in showing responses to environmental conditions. The leaves reflect the morphological alterations as consequences of important environmental changes (Trewavas, 2003). Leaf epidermal studies are of immense significance in finding taxonomy of closely related species. Taxonomists have given prime importance to leaf epidermal features to resolve taxonomic conflicts (Taia, 2005). Both species present thick epidermis being really striate in *Z. coco*. As well it has been informed that cuticle strias are under a strong genetic control (Ahmad, 1962). It is also considered that its development as the increase in thick of the epidermis, it can be influenced by environmental factors. In most of xerophytes, the epidermis presents different kind of strias while in species of mesophytes and hygrophytes the epidermis is plain (Dunn *et al.*, 1965; Wilkinson, 1988). As well, it has been indicated that a thick epidermis contributes a protection of the photosynthetic tissues of a high sun radiation by an increase of the reflectance and striations can interfere in the absorption of the light by the mesophyll chloroplasts (Roth, 1990). In both species the walls of epidermic cells tend to be straight and thick, this characteristic could be associated to aridity conditions and/or to a bigger sunlight exposition (Wilkinson, 1988). By other hand the anticlinal walls of the cell of the abaxial epidermis always present more undulations than in the adaxial surface, this is coincident with what observed by Stace (1965) and agree with Roth (1990) who expresses that this characteristic is due to high shadow and humidity conditions. Both species present predominantly anomocytic stomata, this

was also observed in an analysis of twelve species of the genus *Zanthoxylum* (Xochicale *et al.*, 2010). Stomatal arrangement and types are considered best taxonomic criteria and provide efficient basis for relationship in taxonomic hierarchy (Hameed *et al.*, 2008). Some workers like Sen & Hennipman (1981) had the idea that they may not be so an effective tool in taxonomy because of their inconsistent arrangement in epidermises, however stomatal values like stomatal number and stomatal index are of great value in the evaluation of leaf origin crude drugs (Evans, 2002; Barkatullah *et al.*, 2014). Also, according to Wilkinson (1979) the values of SI are diagnostic features of importance in Systematics, because they not present alterations. However in this work, this affirmation is valid just for *Z. coco*, where the found value of SI (8.03) is similar to the SI (7.65) found in plants of *Z. coco* inhabiting the Yungas in northwestern Argentina (Arambarri *et al.*, 2009). In *Z. armatum* we found a SI= 22.4, which is higher than the value of SI= 15, found in plants of *Z. armatum* from Thailand by Suksathan *et al.* (2009). Kürschner *et al.* (1998) and Salas *et al.* (2001) express that the value of SI is a parameter which is affected by stress conditions, specially nutritional and environmental ones. This allows us to determine that *Z. armatum*, which is exotic in Chaco biogeographical province, could be suffered remarked epidermal changes as result to adaptation to the xeric conditions of chacoan environment. Also we found that *Z. armatum* presents higher stomata density, that it would be possible, under hydric conditions, a higher gaseous exchange than *Z. coco*. Adaptation skills of plants depend on stomatal arrangement on the epidermises, as transpiration and photosynthesis are closely related to stomata. These are also useful in

taxonomic categorization and detection of future clues for observing environmental factors. Micro and macro elements in plants are also closely related to stomatal density (Nabin *et al.*, 2000; Brownlee, 2001). Very little work has been done on the stomatal study of family Rutaceae, Ogunkunle & Olatidele (1997) reported paracytic, hemiparacytic, brachy paracytic, brachy paratetracytic and anomocytic stomatal complexes with uniform size from abaxial epidermises of various *Citrus* species (Rutaceae) and also proved that in spite of high stomatal density, they have relatively low transpiration rate as compared to species with low stomatal density. During a dry season, the margin of the leaves of *Z. armatum* are bended in direction to the abaxial area; this curving can also be suffered by the wings of the rachis. Therefore the appearance of the leaves changes, the stomata are protected, an attenuate diffusion gradient between chlorenchyma and the environment is generated and permits the reduction of stomatal transpiration. This strategy allows the plants can use water efficiently so they are tolerant when this resource is limited (Fahn & Cutler, 1992).

As well the curving of the leaves is an especial answer to the hydric's stress conditions that are observed in many species (Stevanovic, 1986; Stevanovic *et al.*, 1992, Zivkovic *et al.*, 2005), it is also related with a strategy for reducing the foliar area to an important sun radiation (Bocher, 1981). *Z. coco* does not present this behaviour and it is different from *Z. armatum* because shows a bigger development of the palisade parenchyma. This could indicate that it is better for high intensities of light of the region, due to a higher development of palisade parenchyma is related with habitats that are exposed to a drought and intense sun radiation (Bosa-

balidis & Kofidis, 2002; Fahn, 1964). It is necessary to highlight that *Zanthoxylum coco* is native of the northern and central areas of Argentina while *Zanthoxylum armatum* has recently been reported as naturalised (Arana and Oggero, 2009) and both species are found in the same habitat. Although the tissues organization in the stem is similar in both species, the stem of *Zanthoxylum armatum* shows a great capability to store starch in cells of the medulla, xylem and cortex as showed by the stained method used in this work. This species presents a small number of glandular trichomes and secretory cavities that are observed only on the margin of the leaves and the alate rachis, while in *Z. coco* the secretory conducts were very numerous in every analyzed organs and besides in this species it has been found glands in both foliar epidermis.

All these structures are common of observing in many species of Rutaceae (Judd *et al.*, 2008) and it has considered that can contribute to the general regulation of transpiratory rate moderating any hydric unbalance. Kakic *et al.* (2009) studied *Z. acanthopodium* DC., and informed that the type of secretory structure and the secreted substances by this species and the members of this genus must be considered such as ecological meaningful systems of protection and defence that has a plant, since they are important for the taxonomic diagnostic (Metcalfe & Chalk, 1950) and also parameters of the hydric conditions.

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LITERATURE CITED

- Abbasi, A.M.; M.A. Khan, M. Ahmad, M. Zafar, S. Jahan, y S. Sultana, 2010. "Ethnopharmacological application of medicinal plants to cure skin diseases and in folk cosmetics among the tribal communities of North-West Frontier Province". *Pak. J. Ethnopharmacol.*, **128**(2): 322-335.
- AGP III, 2009. "An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III". *Bot. J. Lin. Soc.*, **161**: 105-121.
- Ahmad, K.J., 1962. "Cuticular striations in *Cestrum*". *Curr. Sci.*, **31**: 388-390.
- Arambarri, A.M.; S. E. Freire, N.D. Bayón, M.N. Colares, C. Monti, M.C. Novoa, y M. Hernández, 2009. "Morfoanatomía foliar de árboles medicinales de la Provincia Biogeográfica de las Yungas (Argentina)". *Bol. Lat. Caribe Plant. Med. y Aromát.*, **5**: 342-379.
- Arana, M.D., y A.J. Oggero, 2009. "*Zanthoxylum armatum* (Rutaceae), su presencia en Argentina". *Darwiniana*, **47**(2): 335-338.
- Arshad, M., y M. Ahmad, 2004. *Medico-Botanical Investigation of Medicinally Important Plants from Galliyat Areas, NWFP, Pakistan*. Ethnobotanical Leaflets: 2004(1): Article 6. Available at: <http://opensiuc.lib.siu.edu/eb1/vol2004/iss1/6>.
- Barkatullah, M. I., y N. Muhammad, 2011. "Evaluation of *Zanthoxylum armatum* DC for *in-vitro* and *in-vivo* pharmacological screening". *African Journal of Pharmacy and Pharmacology*, **5**(14): 1718-1723.
- Barkatullah, M.I.; G. Jelani, y I. Ahmad, 2014. "Leaf, Stem Bark And Fruit Anatomy of *Zanthoxylum armatum* Dc. (Rutaceae)". *Pak. J. Bot.*, **46**(4): 1343-1349.
- Babalola, K.A., y A.A. Victoria, 2009. "Foliar Epidermal Morphology of Two West African Genera of Haloragaceae R. BR. (Saxifragales)". *J. Sci. Res. Dev.*, **11**: 84-91.
- Battista, S.M.; G.F. García, A.F. Vugin, A. A. Gurni, y M.L. Wagner, 2007. "Estudio Preliminar de actividad antimicótica en *Fagara coco* (Gillies), Engl – Rutaceae". *Bol. Lat. Caribe Plant. Med. y Aromát.*, **6**(6): 328-329. Universidad de Santiago de Chile. Santiago, Chile.
- Böcher, T.W., 1981. "Evolutionary trends in Ericalean leaf structure". *Det Kon. Danske Vid. Selskab Biol. Skrifter*, **23**: 1-64.
- Bosabalidis, A.M., y G. Kofidis, 2002. "Comparative effects of drought stress on leaf anatomy of two olive cultivars". *Pl. Sci.*, **163**: 375-379.

- Cronquist, A., 1992. *An integrated system of classification of flowering plants*. Columbia University Press, New York. 1262 pp.
- Cutter, E.G., 1969. "Plant Anatomy: Experiment and Interpretation". Part I. *Cell and Tissue*. London; Edward Arnold Press. 157 pp.
- Dunn, D.B.; G.K. Sharma, y C.C. Campbell, 1965. "Stomatal patterns of dicotyledons and monocotyledons". *Amer. Mid. Nat. J.*, **74**: 185-195.
- Escalante, M.G., 1961. "El género *Fagara* en Argentina". *Bol. Soc. Argent. Bot.*, **9**: 291-317.
- Fahn, A., 1964. "Some anatomical adaptations of desert plants". *Phytomorphology*, **14**: 93-103.
- Fahn, A., y D.F. Cutler, 1992. *Xerophytes*. Gebrüder Borntraeger, Berlin. 176 pp.
- Figueiredo-Melo, M.F., y C.S. Zickel, 2004. "Os gêneros *Zanthoxylum* L. e *Esenbeckia* Kunth (Rutaceae) no Estado de Pernambuco, Brasil". *Acta Bot. Bras.*, **18**(1): 73-90.
- Johansen, D.A., 1940. *Plant Microtechnique* VI-VII. Mc- Graw-Hill Book, New York. 523 pp.
- Judd, W.S.; C.S. Campbell, E.A. Kellogg, P.F. Stevens, y M.J. Donoghue, 2008. *Plant Systematics: a phylogenetic approach*, 3rd. ed., Sinauer Assoc, USA. 611 pp.
- Iqbal, I., y M. Hamayun, 2005. "Studies on the Traditional Uses of Plants of Malam Jabba Valley, District Swat", Pakistan Ilyas Iqbal and Muhammad Hamayun thnobotanical Leaflets, (2005)1: Article 32. Available at: <http://opensiuc.lib.siu.edu/ebl/vol2005/iss1/32>.
- Kürschner, W.; I. Stulen, F. Wagner, y P. Kuiper, 1998. "Comparison of palaeobotanical observations with experimental data on the leaf anatomy of durmast oak (*Quercus petraea*) (Fagaceae) in response to environmental changes". *Ann. Bot.*, **81**: 657-664.
- Metcalfe, C.R., y L. Chalk, 1950. *Anatomy of the Dicotyledons - leaves, stem and wood in relation to taxonomy with notes on economic uses*. Clarendon Press, Oxford. 806 pp.
- Morrone, J.J., 2001. *Biogeografía de América Latina y el Caribe*. Manuales & tesis SEA. Zaragoza, España. 150 pp.
- Morrone, J.J., 2004. "La zona de transición sudamericana: caracterización y relevancia evolutiva". *Acta Entomol. Chil.*, **28**: 41-50.
- Nancy, G., y N.G. Dengler, 2002. "An integral part of botany". *Amer. J. Bot.*, **89**: 369-374.
- Rakić, T.; J. Šinžar-Sekulić, B. Filipović, V. Tadić, B. Stevanović, y K. Tan, 2009. "Ecophysiological and anatomical characteristics of the subtropical shrub *Zanthoxylum acanthopodium* (Rutaceae) in conditions of a temperate

- continental climate (Serbia)". *Arch. Biol. Sci. Belgrade*, **61**(2): 249-260.
- Roth, I., 1990. "Peculiar surface structures of tropical leaves for gas exchange, guttation, and light capture (fissures in the epidermis, lenticels, giant stomata, glands, ocelli, papillas, and surface sculpturing)". Rollet, B., C. Högermann, e I. Roth (Eds.). *Stratification of Tropical Forest as seen in Leaf Structure* (Part 2), Kluwer Academic, Amsterdam, Holand. 185-238.
- Salas, J. A.; M.E. Sanabria, y R. Pire, 2001. "Variación en el índice y densidad estomática en plantas de tomate (*Lycopersicon esculentum* Mill.) sometidas a tratamientos salinos". *Bioagro*, **13**: 99-104.
- Stevanović, B., 1986. "Ecophysiological characteristics of the species *Ramonda serbica* Panč. and *R. nathaliae* Panč. et Petrov". *Ekologia* (Belgrade), **21**: 119-134.
- Stevanović, B.; A.T. Pham Thi, F.M. Monteiro de Paula, y J.B. Vieira da Silva, 1992. "Effects of dehydration and rehydration on the polar lipid and fatty acid composition of *Ramonda species*". *Canad. J. Bot.*, **70**: 107-113.
- Seo, M.N., y C. Xifreda, 2008. "Rutaceae". Anton, A.M., Zuloaga, F.O. (Eds), *Flora Fanerogamica Argentina Proflora*, CONICET. **106**: 1-22.
- Stace, C. A., 1965. "Cuticular studies as an aid to plant anatomy". *Bull. British Mus. - Nat. History: Bot.*, **4**: 1-78.
- Stace, C.A., 1984. "The taxonomic importance of the leaf surface". (Eds.): V.H. Heywood and D.M. Moore. *Current Concepts in Plant Taxonomy*. Academic Press, London, **25**: 67-94.
- Suksathan, R.; C. Trisonthi, P. Trisonthi, y P. Wangpakapattanawong, 2009. "Notes on Spice Plants in the Genus *Zanthoxylum* (Rutaceae) in northern Thailand". *Thai Forest Bull. (Bot.)* spec. issue: 197-204.
- Trewavas, A., 2003. "Aspects of Plant Intelligence". *Ann. Bot.*, **92**: 1-20.
- Wilkinson, H.P., 1988. "The plant surface (mainly leaf)". Metcalfe, C.R, y L. Chalk (Eds). *Anatomy of the Dicotyledons*, vol. 1, 2nd ed. Clarendon Press, Oxford, pp. 97-165.
- Xochicale, L.; E. Cocolletzi, y A.R. Andrés-Hernández, 2010. "Análisis anatómico foliar de doce especies del género *Zanthoxylum* L. (Rutaceae)". X Congreso Nacional de Microscopía-Morelia.
- Zhang, D., y T.G. Hartley, 2008. "*Zanthoxylum*". Wu, Z.Y., y P.H. Raven, (Eds). *Flora of China*. Science Press, Beijing, St. Louis, Miss. Bot. Garden, **11**: 52-63.
- Živković, T.; F.M. Quartacci, B. Stevanović, F. Marinone, y F. Navari-Izzo, 2005. "Low-molecular-weight substances in the poikilohydric plant *Ramonda serbica* during dehydration and rehydration". *Plant Sci.*, **168**: 105-111.

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