

Taxonomic Determination of Therapeutic Argentine

Species of *Ilex*

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Abstract

This is comparative study of *Ilex paraguariensis* St. Hil. var. *paraguariensis* Loes., and cogenetic Argentine adulterants: *I. dumosa* Reissek var. *dumosa* Reissek, *I. dumosa* var. *guaranina* Loes., *I. brevicuspis* Reissek, *I. theezans* Reissek and *I. argentina* Lillo. The anatomy and quantitative micrographic parameters (proportional magnitudes, stomata index and palisade ratio) of these leaves was studied to the establish standards in agreement with the WHO for the vegetables drugs. A micrographic parameters table is presented for control quality of the raw material, fragmentary or powdered.

Keywords: Anatomy, “herbal maté”, *Ilex argentina*, *Ilex brevicuspis*, *Ilex dumosa* var. *dumosa*, *Ilex dumosa* var. *guaranina*, *Ilex paraguariensis* var. *paraguariensis*, *Ilex theezans*, micrographic parameters, quality control.

Introduction and objectives

The present investigation was to establish the histological diagnostic characteristics present in the leaf of *Ilex paraguariensis* St. Hil. var. *paraguariensis* “yerba mate” (*Aquifoliaceae*) as well as those of the species that grow in the country and constitute its most conspicuous cogenetic adulterants: *Ilex argentina* Lillo, *Ilex brevicuspis* Reissek, *Ilex dumosa* Reissek var. *dumosa*, *Ilex dumosa* Reissek var. *guaranina* Loesener and *Ilex theezans* Reissek.

The foliar organ constitutes the major raw material used in greater proportion, together with the petioles and the stems. The addition of variable amounts of leaves from the adulterants mentioned not only modify the physiological and pharmacological activities but also induces fluctuations in the content of certain compounds (Pires et al., 1997). This could determine the variation of the traditional characteristics of the “mate” drink and the medicinal preparations

that are used as stimulants, hypotensors (Mazaferra, 1994), diuretics, anti-rheumatics (British Herbal, 1983; Muccillo Baisch et al., 1998).

Past scientific studies of aqueous extracts prepared in form of an infusion allowed verification of the antioxidant action of *Ilex paraguariensis* St. Hil. var. *paraguariensis* (Graham, 1984; Gugliucci, 1996; Schinella et al., 2000).

Quantitative variation of the chemical components of *Ilex* species described by different authors (Pires et al., 1997) justifies the need for botanical identification of each pharmaceutical constituent. Thus, in the present work, the stomata index and palisade ratio were obtained, also from a study of the foliar anatomy that identifies the raw drug fragmented or pulverized allowing the establishment of the taxonomic status of the species involved and facilitating its quality control.

Materials and methods

The studied material was constituted by fresh specimens from the Experimental Station of the INTA Cerro Azul (Misiones). The samples of the employed material are deposited in the Herbario del Museo de Botánica y Farmacognosia “Carlos Spegazzini” of the Facultad de Ciencias Exactas of La Plata U.N.L.P. (LPE).

The analyzed specimens belong to the following institutions: Herbario CEFYBO, Consejo Nacional de Investigaciones Científicas y Técnicas (BACP). Herbario “Juan A. Domínguez” Museo de Farmacobotánica, Facultad de Bioquímica y Farmacia, UBA (BAF). Herbario División Plantas Vasculares, Museo de Ciencias Naturales de La Plata, U.N.L.P. (LP). Herbario “Carlos Spegazzini” de la Facultad de Ciencias Exactas, U.N.L.P. (LPE). Herbario Instituto de Botánica Darwinión (SI).

Ilex paraguariensis* var. *paraguariensis

ARGENTINA. Prov. Buenos Aires: A.M. Fuchs (SI 16642, leaves a and b). Prov. Misiones: A. Burkart 28405 (SI); Zardini et al. 727 (LP); C. Spagazzini, 26/1/1907 (LP); S. Prat Kricum (LPE 938); Boelcke et al. 5421 (SI); J.C. Gamberro et M. Toursarkissian 151 (SI); J.F. Molfino, (LPE 87, 93); A.L. Cabrera et al. 28906 (SI); V. Maruñak 194 (SI); G.C. Giberti et O. Ahumada 3 (SI); A. Schinini et al. 24281 (BACP); G.C. Giberti et Daviña 199 (BACP); J.F. Molfino (LPE 96, 97); M.I.H.S. de Birabén, 10/1969 (LP); unknown collector, 104 1/07 (LP); I.C. Vattuone et A. Bianchi, 6-3/1914 (BAF 191-201-202-205); León Denis n° 2,4,5,6,7 (BAF); Gozulbo V. n° 1 (BAF); San Ignacio 17/1/1914,4,5,6,7,8 (BAF); S. Prat Kricum (LPE 970, 976).

URUGUAY. Corn Osten: n° 19689 (BAF); unknown collector n° 19690 (BAF).

PARAGUAY. T. Rojas n° 4020 (BAF); T. Rojas, 1916 (BAF). BRASIL. G.C. Giberti et al. 328 (BACP); G.C. Giberti et al. 393, 394 (BACP); G.C. Giberti et al. 397 (BACP); G.C. Giberti et al. 405 (BACP); G.C. Giberti et al. 411 (BACP); G.C. Giberti et al. 419 (BACP); G.C. Giberti et al. 423 (BACP); S. Prat Kricum et al. 225 (BACP); A. Lehnm 2204 (SI).

Ilex argentina

ARGENTINA. Prov. Jujuy: Cabrera et al. 32020, 32046 (SI); A.L. Cabrera et H.A. Fabris 21130 (LP); unknown collector, 11/1911 (LP). Prov. Misiones: S. Prat Kricum (LPE 971, 977). Prov. Salta: Lázaro Novara 3809 (LP). Prov. Tucumán: S. Venturi 9990 (LP); Descole et al., ex-LIL 35169 (LP); S. Venturi 4623 (SI); G.C. Giberti s/n° (BACP 2828).

Ilex brevicuspis

ARGENTINA. Prov. Misiones: T. Rojas 4488 (LP); G.C. Giberti et S.D. Prat Kricum 355 (BACP).

BRASIL. F.C. Hoehne 28597 (LP); B. Rambo 29438 (SI); G.C. Giberti et al. 298, Prat Kricum (LPE 973, 978); G.C. Giberti et al. 304, 308 (BACP).

Ilex dumosa* var. *dumosa

ARGENTINA. Prov. Misiones: Prat Kricum (LPE 960, 972).

BRASIL. G.C. Giberti, S.D. et Prat Kricum, D. Da Croce et P. Floss 237, 238 (BACP).

Ilex dumosa* var. *guaranina

ARGENTINA. Prov. Corrientes: A. Schinini et al. 11165 (SI). Prov. Misiones: A. Schnander 10248 (SI); S. Prat Kricum (LPE 979) (BAF) X/ 1977.

PARAGUAY. E. Migone 10599 (SI); Pavetti et Rojas 10048 (SI).

Ilex theezans

ARGENTINA. Prov. Misiones: S. Prat Kricum (LPE 975, 976); S. Prat Kricum et al. 54 (BACP,SI).

BRASIL. G.C. Giberti, et al. 297, 302 (BACP); G.C. Giberti, et al. 310 (BACP); G.C. Giberti, et al. 427 (BACP).

Dry material was used for the observations of the foliar epidermis with an optic microscope, proceeding from its diaphanization and elimination of the cutina by a technique created *ad hoc* (Carpano et al., 1994), by which cells could be visualized and proportional numerical values could be obtained: stomata index (Salisbury, 1927) and palisade ratio (Zornig & Weiss, 1925). The transversal cuts of the leaves were practiced with a Ranvier microtome, from fresh material fixed in absolute alcohol: glacial acetic acid (3 : 1) or dry and hydrated in heat. A part of the same was diaphanized with 10% NaOH and another was colored with saphranine-fast green (D'Ambrogio, 1986; Dizeo, 1979). The cuts were mounted on synthetic balsam Biopur, becoming part of the collection of each species. The microchemical tests were practiced *in situ* with the following reagents: hydrochloric phloroglucin, hydrochloric acid and methylene blue in order to establish lignin, calcium oxalate and mucilage, respectively.

The observations were performed with an Olympus CH optical microscope as well as the original designs, equipped with an Iroscope design tube. The symbols used are those of Metcalfe and Chalk (1957).

Results

Ilex paraguariensis St. Hil. var. *paraguariensis*

In: Mem. Mus. Hist. Nat. 9: 351, 1822.

Syn.: *Ilex paraguariensis* f. *latifolia* Chodat, *Ilex paraguariensis* f. *parvifolia* Chodat (Grondona, 1954)

c.n.: "herbal mate", "yerba mate", "árbol del mate", "Caá", "té del Paraguay", "yerba señorita", "palo de yerba mate", "caá-guazú".

Epidermal characters of the leaf:

- Thick and ornamented cuticle.
- Adaxial epidermis with cells with straight contours (Fig. 1A).
- Abaxial epidermis with cells with slightly undulated contours (Fig. 1B).
- Unicellular non-glandular trichomes in both epidermis.
- Cyclocytic stomata in the abaxial epidermis (Fig. 1B).
- Abundant hydathodes in the abaxial epidermis (Fig. 1C).

Anatomical characters of the transverse section:

- Dorsiventral or bifacial structure (Fig. 1D).
- Very thick and ornamented cuticle.
- Unistratified adaxial epidermis, interrupted by cells where periclinal divisions are produced and in which mucilaginous content appears (Fig. 1D).

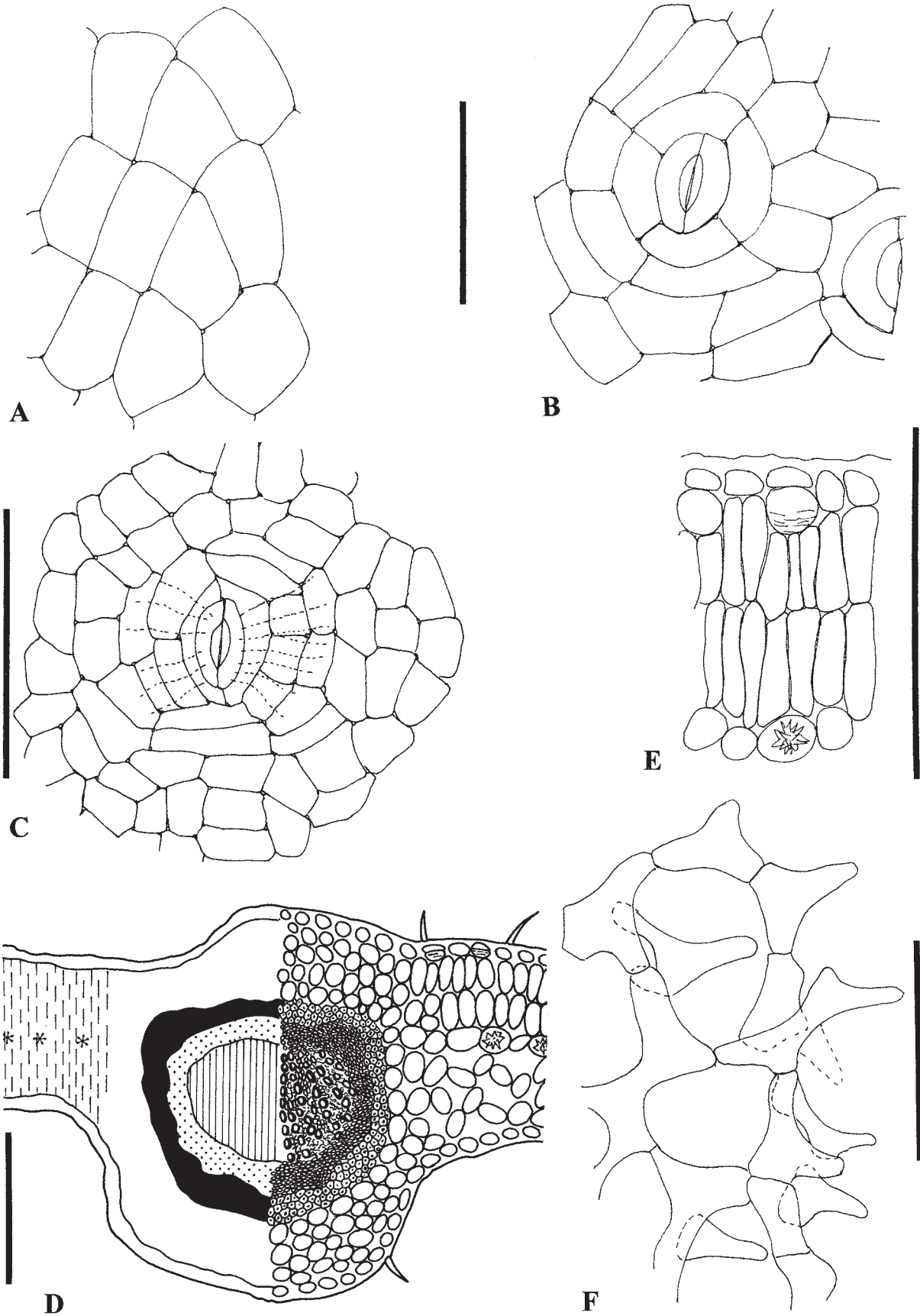


Figure 1. *I. paraguariensis* var. *paraguariensis*. Leaf: Epidermis design: A- adaxial, B- abaxial, C- hydathode. Transverse section: D- Main nerve design, E, F- Parenchyma in palisade and spongy design, respectively. Scale (dark bar) = 100 μ m.

- Mesophyll: parenchyma in palisade with cells in three layers. The height of the same is the same as the first two lowering the third towards the internal zone of the mesophyll (Fig. 1D, E, F).
- Braciform spongy parenchyma (Fig. 1F).
- Crystalline substances: calcium oxalate druses in the mesophyll cells (Fig. 1D, E).
- Vascular system of the main nerve: presence of amphivasal sheaf surrounded by a sheath complete with sclerenchymatic fibers (Fig. 1D).
- Unistratified abaxial epidermis with a thick cuticle interrupted by stomata and hydathodes.

Quantitative Analytical Microscopy

Stomata index: 6.89 (10.13) 15.50.

Palisade ratio: 2.50 (3.00) 4.50.

Ilex argentina Lillo

In: *Anales Soc. Cient. Argent.* 72:171, 1911.

c.n.: “árbol de la yerba”, “palo de yerba”, “naranjillo”, “roble”, “roble de Tucumán”, “roblina”.

Epidermal characters of leaf:

- Ornamented cuticle.
- Adaxial epidermis with cells of straight contours, some present periclinal or anticlinal divisions (Fig. 2A).
- Abaxial epidermis with cells with undulated contours, some with thick and lignified walls (Fig. 2B).
- Unicellular non-glandular trichomes in both epidermis.
- Cyclocytic or bicyclic stomata in the abaxial epidermis (Fig. 2B).
- Very scarce hydathodes in the abaxial epidermis.

Anatomical characters of the transverse section:

- Dorsiventral or bifacial structure (Fig. 2C, D).
- Very thick and ornamented cuticle.
- Unistratified adaxial epidermis with some cells of greater height that penetrate in the parenchyma in palisade (Fig. 2C, D).
- Mesophyll: cells of the parenchyma in palisade in two layers, one of greater height and the shorter one towards the center of the mesophyll; braciform spongy parenchyma towards the abaxial epidermis (Fig. 2C, D).
- Crystalline substances: calcium oxalate druses, in the spongy parenchyma (Fig. 2C, D).
- Vascular system of the main nerve: constituted by a collateral sheaf with a cap of sclerenchyma towards the abaxial epidermis and collenchyma towards the adaxial epidermis. Complete sheath of the sheaf, formed by parenchyma cells (Fig. 2C).
- Unistratified abaxial epidermis with a thick cuticle, interrupted by stomata and scarce hydathodes.

Quantitative Analytical Microscopy

Stomata index: 6.60 (12.38) 20.00.

Palisade ratio: 6.00 (7.95) 11.50.

Ilex brevicuspis Reissek

In: C. Martius, *Fl. Bras.* 11 (1) 56, 1861.

Syn.: *Ilex caaguazuensis* Loesener (Giberti, 1979).

c.n.: “Cauna”, “caona”, “mico”, “ornhela de mico”, “cauna de monte”, “siete sangrías”.

Epidermal characters of the leaf:

- Ornamented cuticle.
- Adaxial epidermis with cells with undulated contours (Fig. 3A).
- Abaxial epidermis with cells with straight contours (Fig. 3B).
- Unicellular non-glandular trichomes in both epidermis.
- Cyclocytic stomata in the abaxial epidermis (Fig. 3B).

Anatomical characters of the transverse section:

- Dorsiventral or bifacial structure (Fig. 3C, D).
- Thick and ornamented cuticle.
- Unistratified adaxial epidermis (Fig. 3C, D).
- Mesophyll: parenchyma cells in palisade in two layers, braciform spongy parenchyma towards the abaxial epidermis (Fig. 3C, D).
- Vascular system of the main nerve: collateral sheaf, semicircular cap of sclerenchyma towards the abaxial epidermis (Fig. 3C).
- Abaxial epidermis with thick unistratified cuticle with stomata.

Analytical quantitative microscopy

Stomata index: 3.80 (8.22) 11.49.

Palisade ratio: 2.50 (3.57) 4.75.

Ilex dumosa Reissek

In: Martius, *Fl. Bras.* 11 (1) 56, 1861.

Syn.: *Ilex dumosa* var. *montevideensis* Loes.; *Ilex dumosa* var. *gomezii* Loes. (Giberti 1979).

c.n.: “apere’a Ka’a”, “Ka’a mini”.

Ilex dumosa Reissek var. *dumosa*

Syn.: *Ilex dumosa* Reissek var. *montevideensis* Loes.; *Ilex amara* (Vell. Conc.) Loes. var. *latifolia* Reissek f. *microphylla* Reissek ex Loes.; *Ilex dumosa* Reissek var. *mosenii* Loes. (Giberti, 1989).

c.n.: “caá-chirivi”, “caúna dos capões”, “congonha”, “congonha miuda”, “congonha miuda do brejo”, “congonilla”.

Epidermal characters of the leaf:

- Ornamented cuticle.
- Adaxial epidermis with cells with undulated contours (Fig. 4A).
- Abaxial epidermis with cells with slightly undulated contours (Fig. 4B).
- Unicellular non-glandular trichomes in both epidermis.
- Anomocytic to cyclocytic stomata in the abaxial epidermis (Fig. 4B).
- Scarce hydathodes in the abaxial epidermis.

Anatomical characters of the transverse section:

- Dorsiventral or bifacial structure (Fig. 4C, D).
- Very thick and ornamented cuticle.
- Unistratified adaxial epidermis (Fig. 4C, D).

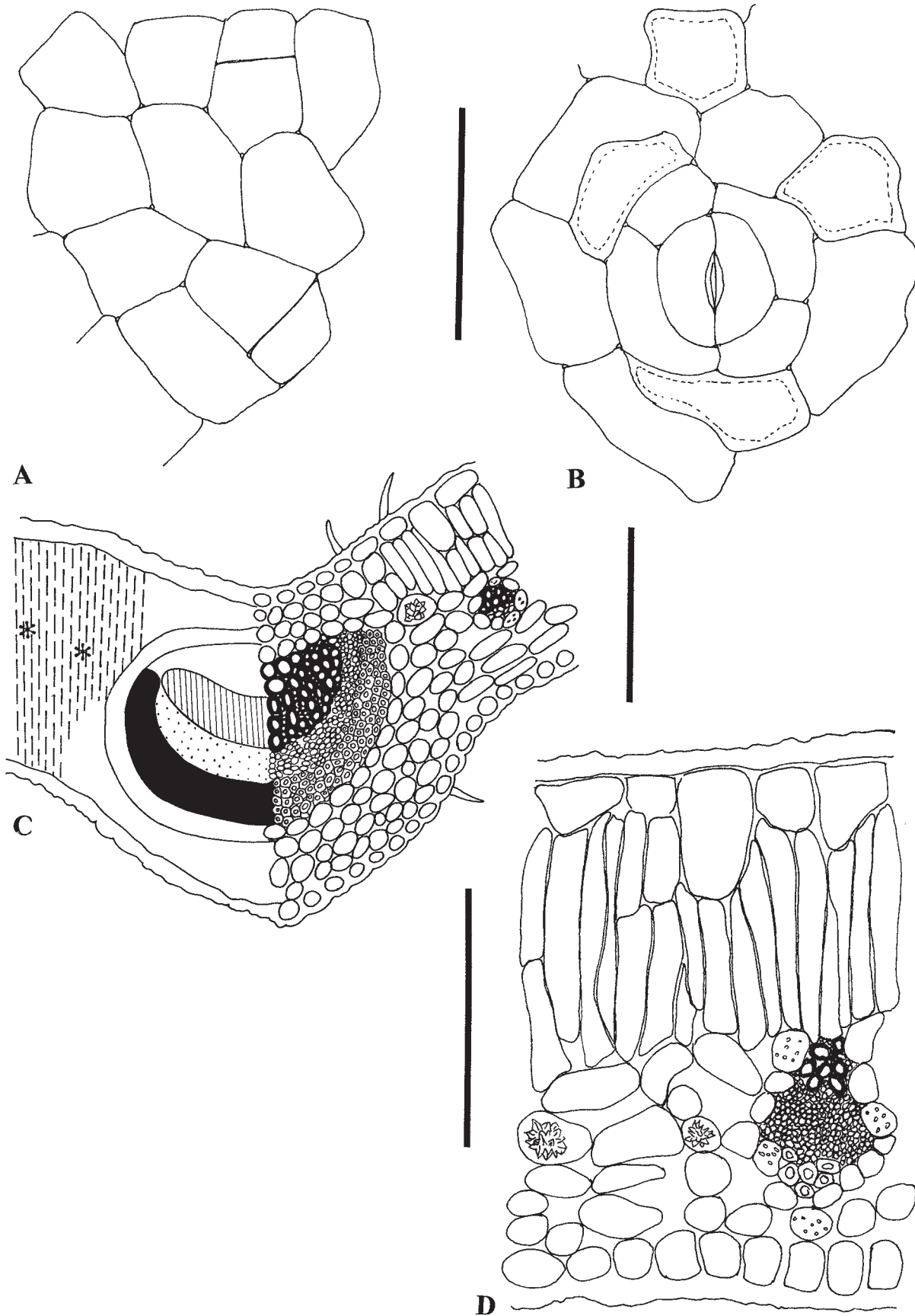


Figure 2. *I. argentina*. Leaf: Epidermis design: A- adaxial, B- abaxial. Transverse section: C- Main nerve design, D- Mesophyll detail. Scale (dark bar) = 100 μ m.

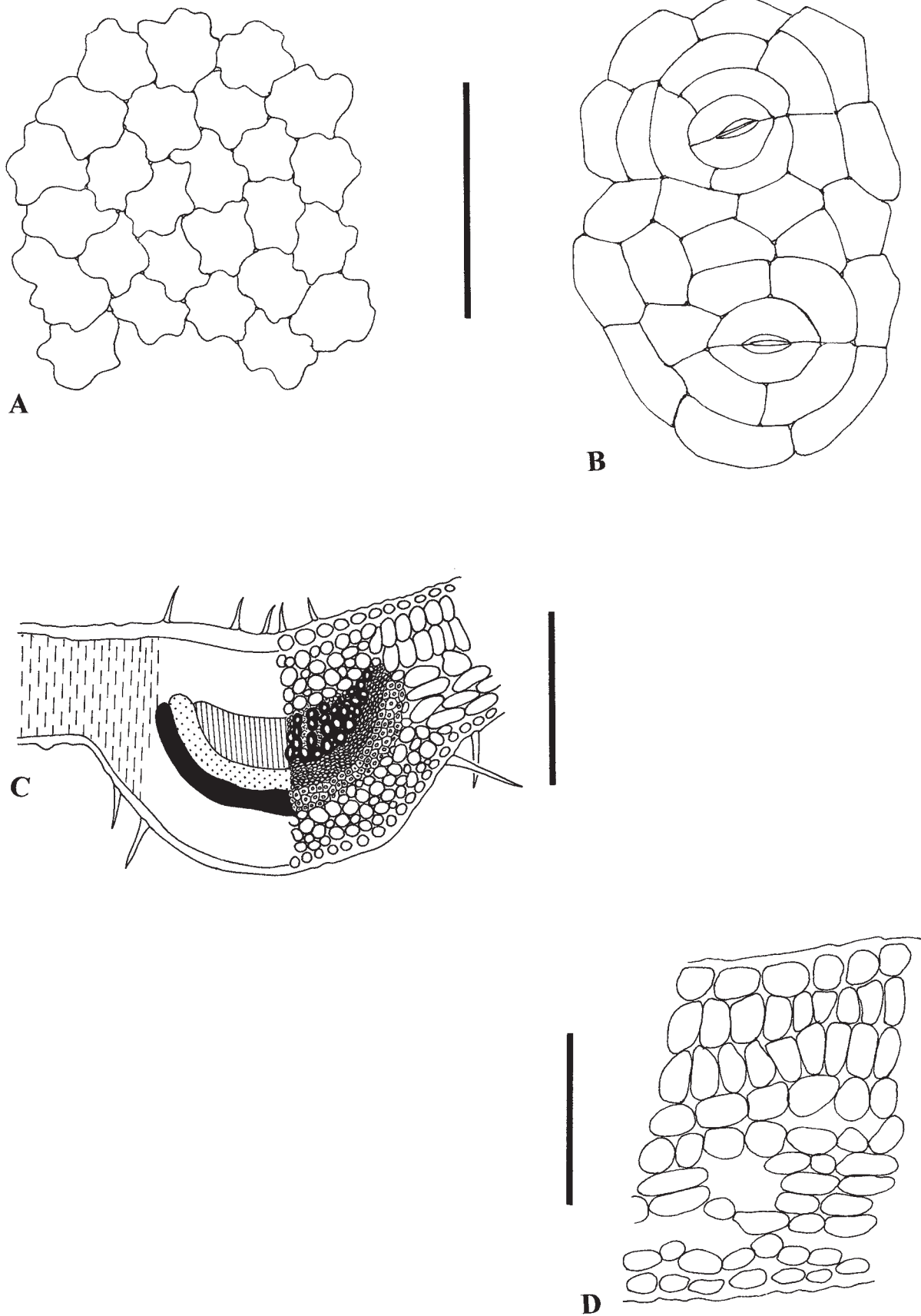


Figure 3. *I. brevicuspis*. Leaf: Epidermis design: A- adaxial, B- abaxial. Transverse section: C- Main nerve design, D- Mesophyll detail. Scale (dark bar) = 100 μm .

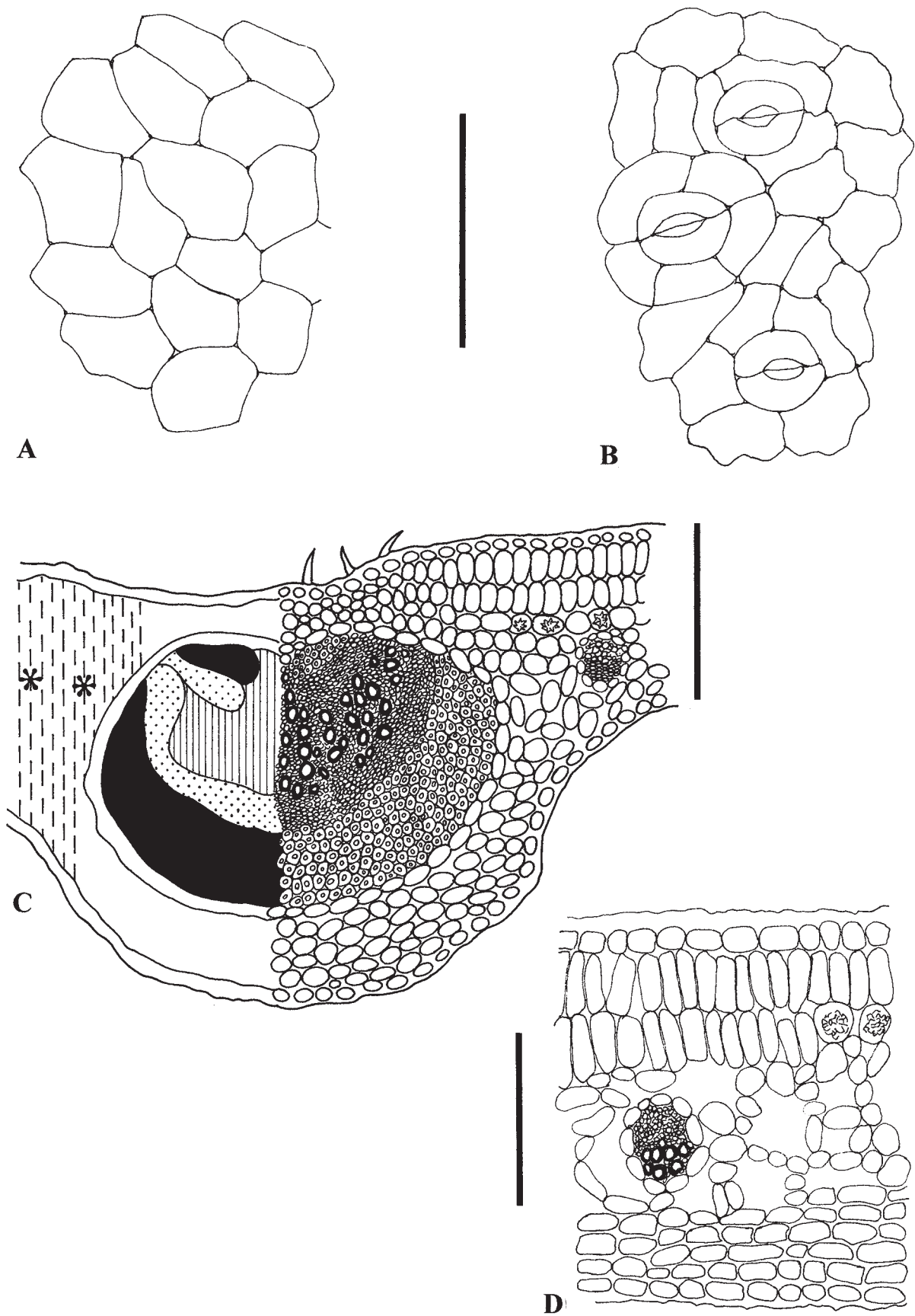


Figure 4. *I. dumosa* var. *dumosa*. Leaf: Epidermis design: A- adaxial, B- abaxial. Transverse section: C- Main nerve design; D- Mesophyll detail. Scale (dark bar) = 100 μm.

- Mesophyll: parenchyma cells in palisades in two layers, bractiform spongy parenchyma towards the abaxial epidermis (Fig. 4C, D).
- Crystalline substances: calcium oxalate druses, in the spongy parenchyma (Fig. 4C, D).
- Vascular system of the principal nerve: constituted by a collateral sheaf with a cap of sclerenchyma and complete sheath of the sheaf formed by parenchyma cells (Fig. 4C).
- Abaxial epidermis with a thick cuticle, stomata and hydathodes.

Analytical quantitative microscopy

Stomata index: 7.36 (9.80) 13.04.

Palisade ratio: 1.25 (2.41) 3.25.

Ilex dumosa Reissek var. *guaranina* Loes.

In: Nova Acta Acad. Caes. Leop. Carol. German. Nat. Cur., 78:198, 1901.

Syn.: *Ilex dumosa* f. *angustifolia* Chodat (Giberti, 1989).

c.n.: “ka’ a ygapo”, “ka’ a la niña”, “guaranina”, “yerba señorita”, “caá-berá”, “caáchiri”, “cauna”.

Epidermal characters of the leaf:

- Ornamented cuticle.
- Adaxial epidermis with cells with undulated contours (Fig. 5A).
- Abaxial epidermis with undulated contours, some with thickened and lignified walls (Fig. 5B).
- Unicellular non-glandular trichomes in both epidermis.
- Anomocytic to cyclocytic stomata in the abaxial epidermis (Fig. 5B).
- Hydathodes in the abaxial epidermis.

Anatomical characters of the transverse section:

- Dorsiventral or bifacial structure (Fig. 5C, D).
- Thick and ornamented cuticle.
- Unistratified adaxial epidermis (Fig. 5C, D).
- Mesophyll: parenchyma cells in palisade in two layers, bractiform spongy parenchyma towards the abaxial epidermis (Fig. 5C, D).
- Crystalline substances: calcium oxalate druses, in the third layer of shorter palisade cells (Fig. 5C, D).
- Vascular system of the main nerve: constituted by a collateral sheaf surrounded by a complete sclerenchymatous cells sheath and towards the abaxial epidermis a collenchyma cap (Fig. 5C).
- Abaxial epidermis with a thickened cuticle, stomata and hydathodes.

Analytical quantitative microscopy

Stomata index: 7.69 (10.93) 13.84.

Palisade ratio: 1.25 (2.31) 4.00.

Ilex theezans C. Martius ex Reissek

In: C. Martius, FI. Bras, 11 (1): 51, 1861.

c.n.: “acebo gigantesco”, “caa-ná”, “caá panami”, “cauna”, “cauna amargosa”, “cauna de folhas largas”, “chá-mate”,

“congonha”, “palo de aceite”, “yerba colorada”, “yerba”, “hierba de aceite” (Giberti, 1990).

Epidermal characters of the leaf:

- Ornamented cuticle.
- Adaxial epidermis with cells of straight contours, some of greater size (Fig. 6A).
- Abaxial epidermis with cells with slightly undulated contours (Fig. 6B).
- Unicellular non-glandular trichomes in both epidermis.
- Diacytic to bicyclic stomata in the abaxial epidermis (Fig. 6B).
- Hydathodes in the abaxial epidermis.

Anatomical characters of the transverse section:

- Dorsiventral or bifacial structure (Fig. 6C, D).
- Thick and ornamented cuticle.
- Pluristratified adaxial epidermis (Fig. 6C, D).
- Mesophyll: parenchyma cells in palisade in four or five layers, bractiform spongy parenchyma towards the abaxial epidermis (Fig. 6C, D).
- Crystalline substances: calcium oxalate druses, in the spongy parenchyma (Fig. 6C, D).
- Vascular system of the main nerve: constituted by a collateral sheaf surrounded by an incomplete sclerenchyma sheath and towards the adaxial epidermis to central collenchymas (Fig. 6C).
- Abaxial epidermis with a thick unistratified cuticle, interrupted by stomatas and hydathodes.

Analytical quantitative microscopy

Stomata index: 6.49 (11.26) 14.79.

Palisade ratio: 1.75 (4.12) 8.75.

Discussion

The data from different authors of the exo- and endomorphological foliage of *Ilex paraguariensis* var. *paraguariensis* and the five cogenetic species, with ours observations. With respect to the stomata, the genus *Ilex* sp., can present different types: cyclocytic in *Ilex paraguariensis*, *Ilex argentina*, *Ilex brevicuspis*, *Ilex dumosa* var. *guaranina* and *Ilex dumosa* var. *dumosa*; bicyclic in *Ilex argentina* and *Ilex theezans*; meanwhile, the anomocytic are only found in *Ilex dumosa* and its varieties and diacytic in *Ilex theezans*, agreeing with that expressed by Baas (1975).

Thus, we do not agree with Jackson and Snowdon (1968) and Metcalfe and Chalk (1957) and that published in the British Herbal (1983) where they indicate that the type of stomata of *Ilex paraguariensis* is anomocytic, being that it was proven that is of the cyclocytic type.

The majority of the investigators do not consider the existence of hydathodes, cork warts or foliar lenticels; on the other hand, it can be established that they possess diagnostic value and are very important in the genus *Ilex*. Its presence has been observed in the abaxial epidermis of the studied

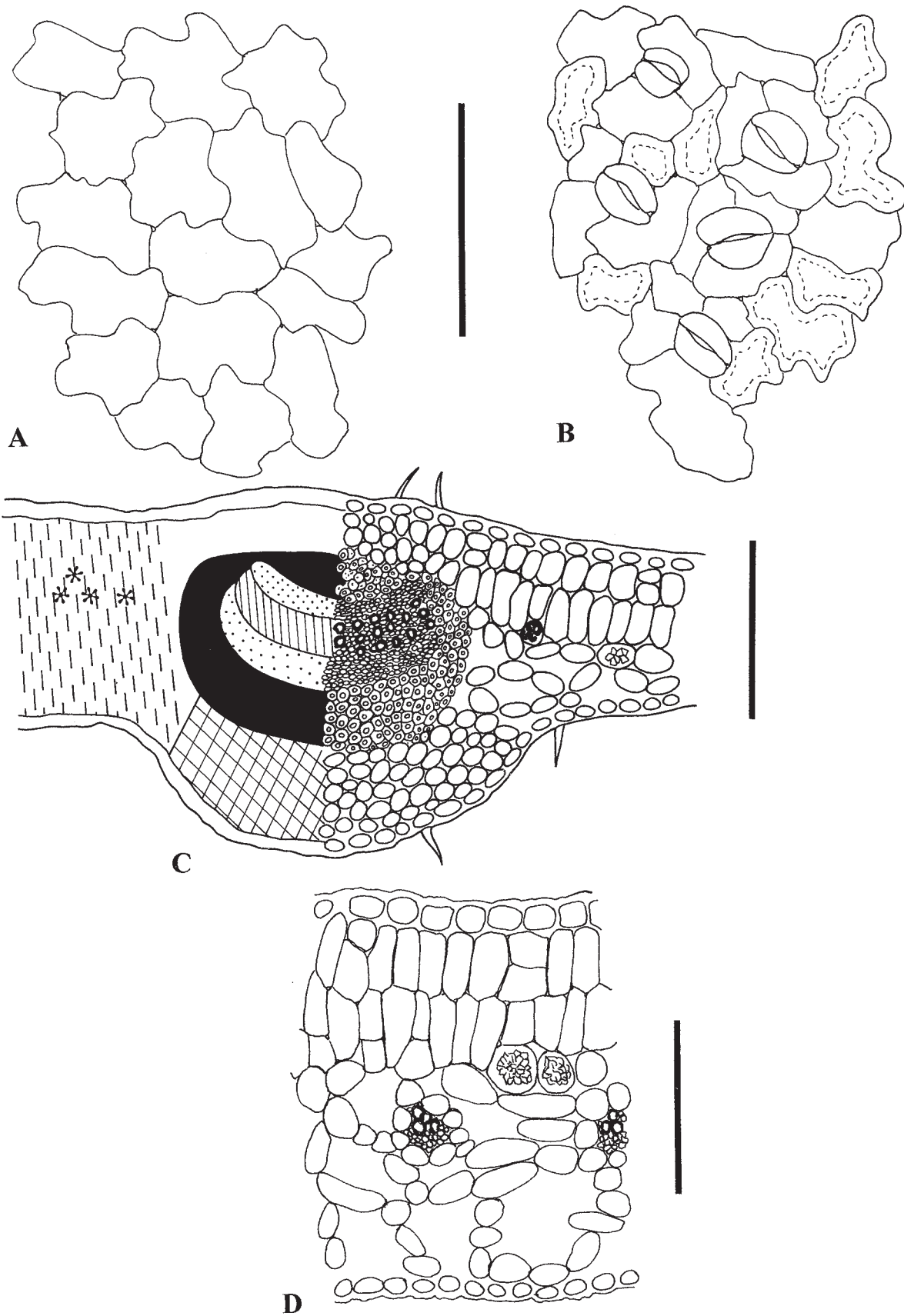


Figure 5. *I. dumosa* var. *guaranina*. Leaf: Epidermis design: A- adaxial, B- abaxial. Transverse section: C- Main nerve design, D- Mesophyll detail. Scale (dark bar) = 100 μ m.

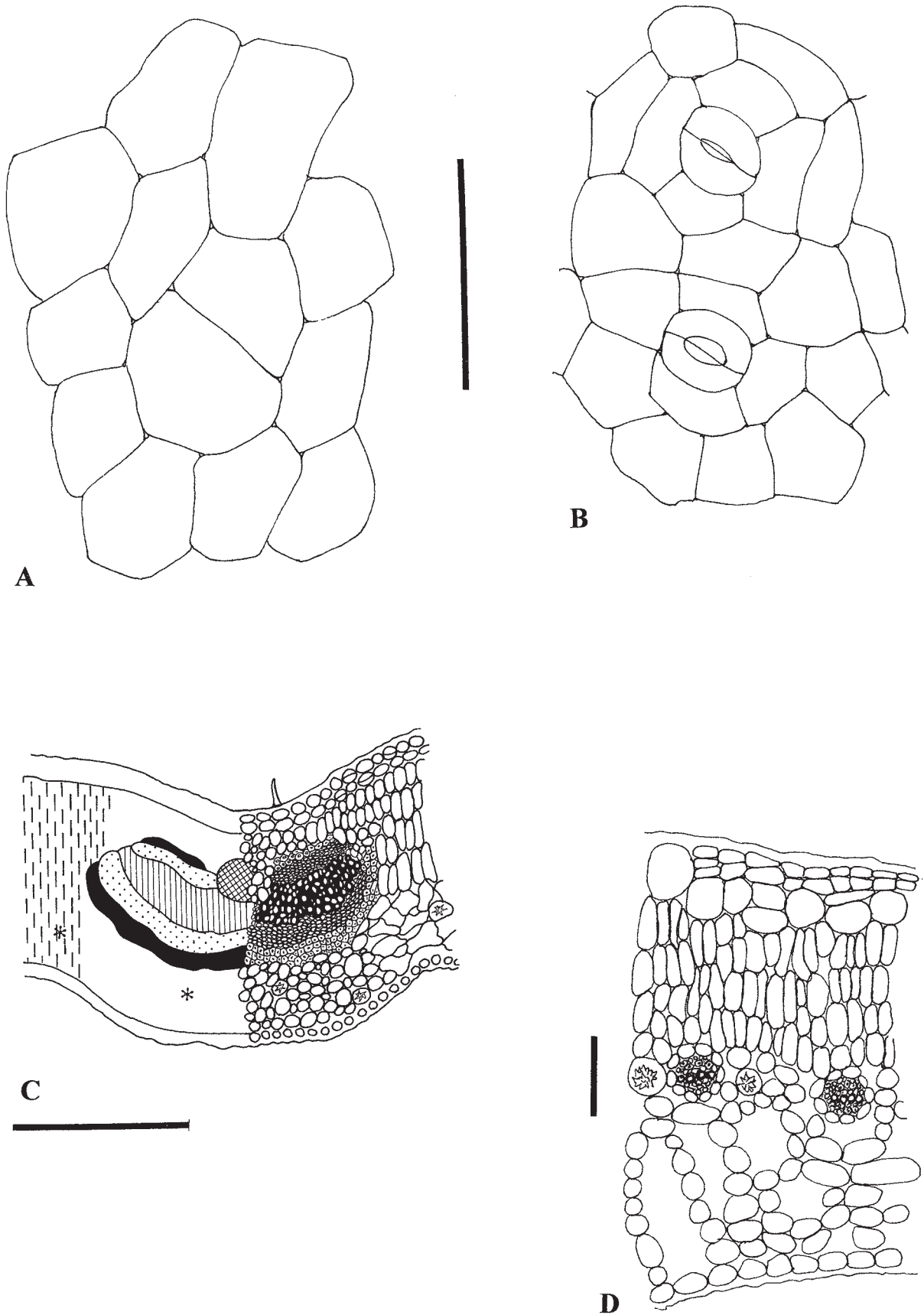


Figure 6. *I. theezans*. Leaf: Epidermis design: A- adaxial, B- abaxial. Transverse section: C- Main nerve design, D- Mesophyll detail. Scale (dark bar) = 100 μ m.

species except in *Ilex brevicuspis*. In *Ilex paraguariensis* var. *paraguariensis* they are abundant; in *Ilex argentina*, *Ilex theezans* and in two varieties of *Ilex dumosa*, they appear in less quantity.

According to Coelho (1995), the existence of foliar lenticels could be a differential characteristic between varieties of *Ilex paraguariensis*, but he only considered *Ilex paraguariensis* var. *sincorensis* Loes. which do not grow in Argentina. Nevertheless, the presence of the same in *Ilex paraguariensis* var. *paraguariensis*, would be detracting from such assertion.

On the other hand, for *Ilex argentina*, the cited author manifest the absence of them, nonetheless we have observed them. With respect to the differential characteristics between the epidermis of the different species and varieties it can be pointed out that the presence of lignified cells in the abaxial epidermis of *Ilex argentina* and *Ilex dumosa* var. *guaranina*, characters of diagnostic value already observed by Lendner (1917), Baas (1975) and Barral et al. (1995).

In relation to epidermal cells, they are differentiated in the different species and varieties; Scala (1921) defined the species by such characters.

The presence of epidermal non-glandular trichomes in these materials was attributed (Oglobin, 1939; Coelho, 1995), to the action of psilidio: *Gyropsylla spegazziniana* L. known as "blister" of the yerba mate and producer of the "curl of yerba mate" (Fernandez Diaz, 1997). Such a concept is erroneous being that a non-glandular trichome is initiated from an epidermal cell. The epidermal unicellular hair present in all species and varieties of *Ilex* studied which are observed at the level of the main nerves in both epidermis, as quoted by Loesener (1901) in his original diagnosis.

Many authors deny their presence (Coppetti, 1916; Scala, 1921), which must be attributed to the handling of the different treatments that the leaves of *Ilex* suffer, in the process of elaboration of "yerba mate". Meanwhile, the fresh material, recently herborized, is observed in such trichomes.

The anatomical characters observed with an optic microscope in all species and varieties of the genus *Ilex* a dorsoventral or bifacial structure is seen that is constituted by parenchyma in palisade towards the adaxial epidermis and a brachiform spongy parenchyma towards the abaxial epidermis. Thick and ornamented cuticles in both epidermis.

The adaxial epidermis in general is unistratified in the different species except in *Ilex theezans*, which appears pluristratified or multiple as expressed by Coelho (1995). Baas (1975) also included mucilaginous idioblasts. According to our analysis, it coincides with both.

Such layer in *Ilex paraguariensis* var. *paraguariensis*, is interrupted by mucilaginous cells, already indicated by Lendner (1917).

Some epidermal cells appear in *Ilex argentina* of greater height which penetrate in the parenchyma in palisade. According to Giberti (1989) they are mucilaginous idioblasts and for Baas (1975) is multiple epidermis, but the observations performed demonstrate the existence of periclinal divi-

sions and the presence of mucilaginous idioblasts coinciding with Giberti. It can be said the *Ilex paraguariensis* var. *paraguariensis* possesses unistratified epidermis presenting some cells with mucilaginous content.

We must conclude, with respect to the abaxial epidermis, that in all the species of *Ilex* studied, a thick cuticle, stomata cells and hydathodes appear. These last histological elements are not observed in the case of *Ilex brevicuspis*.

The number of layers of the parenchyma in palisade is variable. Two cellular layers are observed in *Ilex paraguariensis* var. *paraguariensis* of equal size and a shorter third layer towards the internal zone in contact with the spongy parenchyma. According to Brieger (1995) it presents a layer in palisade, where in Coelho (1995) manifests that a fourth layer in transition exists, eventually.

Ilex argentina e *Ilex brevicuspis* possess one to two layers, *Ilex dumosa* var. *dumosa* two, *Ilex dumosa* var. *guaranina* two to three and in *Ilex theezans* from three to five layers.

Crystal Idioblasts:

The position in the mesophyll of the calcium oxalate druses is variable: in *Ilex paraguariensis* var. *paraguariensis* and *Ilex dumosa* var. *guaranina* they are specially located in the third palisade layer, in *Ilex argentina*, *Ilex dumosa* var. *dumosa* and *Ilex theezans* dispositioned in the spongy parenchyma, and in *Ilex brevicuspis* none are observed. The authors already cited describe the druses but do not define their position.

Vascular System of the main nerve:

An amphyvasal sheaf is observed in *Ilex paraguariensis* var. *paraguariensis* surrounded by an sclerenchymatic sheath. The sheaf in *Ilex argentina* is collateral with a sclerenchyma cap towards the abaxial epidermis and collenchyma towards the adaxial epidermis. The sheath of the sheaf is complete and formed by parenchyma cells.

The sheaf in *Ilex brevicuspis* is collateral, with a semicircular cap of sclerenchyma towards the abaxial epidermis.

The sheaf in *Ilex dumosa* var. *dumosa* is collateral with a basal cap of sclerenchyma and the sheath of the sheaf is formed by parenchyma cells.

The sheaf in *Ilex dumosa* var. *guaranina* is collateral surrounded by a complete sheath of sclerenchyma and towards the abaxial epidermis. It possesses a cap of collenchymatic cells.

The sheaf in *Ilex theezans* is collateral with a semicircular cap of sclerenchyma towards the abaxial epidermis and towards the adaxial epidermis at the extremes that present two caps of phloem over the xyleme and in the center of the sheaf a group of collenchymatic cells appear. The majority of the investigations consulted on these aspects of the anatomy of *Ilex* did not supply data on the subject.

The numerical values obtained by quantitative analytical microscopy provide proportional numerical magnitudes that are original and unpublished.

Conclusions

As expressed by Scala (1921): “The histological study (micrographic) of the ‘yerbas’ is the first and inevitable auxiliary for the secure recognition of the known falsifications and tampering, probable or simply possible. The histological study must be solidary with the chemical study, the alteration in order to establish the official percentage of caffeine in that a yerba micrographically genuine and pure could have been deprived of its main active principle”.

The present investigation contributes the development of an original method for the elimination of the cutina in foliar organs that without the obtainment of numerical values (index) would have been impossible.

The results of the micrographic analysis are applicable to the identification and quality control of *Ilex paraguariensis* var. *paraguariensis* and the cogeneric species: *Ilex argentina*, *Ilex brevicuspis*, *Ilex dumosa* var. *guaranina*, *Ilex dumosa* var. *dumosa* and *Ilex theezans*, since they allow:

- Observation of the cells of the adaxial epidermis with straight contours in *Ilex paraguariensis* var. *paraguariensis*, *Ilex argentina* and *Ilex theezans*; with undulated contours in *Ilex brevicuspis*, *Ilex dumosa* var. *dumosa* and *Ilex dumosa* var. *guaranina*; and also the absence of stomata in all cases, in such epidermis.
- Establish that in the cells of the abaxial epidermis the contours are straight in *Ilex brevicuspis*; slightly undulated in *Ilex paraguariensis* var. *paraguariensis*, *Ilex dumosa* var. *guaranina* and *Ilex theezans*; undulated contours in *Ilex argentina* and *Ilex dumosa* var. *dumosa*.
- Visualize in the abaxial epidermis lignified cells in *Ilex argentina* and *Ilex dumosa* var. *guaranina*.
- Establish the types of stomata present in the abaxial epidermis in each species and varieties: ciclocytic in the species and varieties studied except *Ilex theezans*; bicyclic can be found in *Ilex argentina* and *Ilex theezans*; anomocytic in both varieties of *Ilex dumosa* and diacytic in *Ilex theezans*.
- Determine the presence of non-glandular trichomes in all the species and varieties.
- Confirm the existence of hydathodes, warts and cork warts like lenticels or foliar lenticels in the abaxial epidermis in the species and varieties studied except in *Ilex brevicuspis*.

The transverse sections of each species and variety allowed to define the following characters:

- Unistratified epidermis in *Ilex paraguariensis* var. *paraguariensis*, *Ilex argentina*, *Ilex brevicuspis*, *Ilex dumosa* var. *guaranina* and *Ilex dumosa* var. *dumosa*.
- Pluristratified epidermis in *Ilex theezans*.
- Mucilaginous idioblasts in *Ilex paraguariensis* var. *paraguariensis*, *Ilex argentina*, *Ilex dumosa* var. *guaranina* and *Ilex theezans*.
- Mesophyll with a variable number of layers of palisade cells: one to two in *Ilex argentina* and *Ilex brevicuspis*, two

in *Ilex dumosa* var. *dumosa*, two to three in *Ilex dumosa* var. *guaranina*, three in *Ilex paraguariensis* var. *paraguariensis* and from three to five in *Ilex theezans*.

- Druses are found in the species and varieties studied except in *Ilex brevicuspis*. In *Ilex paraguariensis* var. *paraguariensis* and *Ilex dumosa* var. *guaranina* the same are specially disposed in the third layer of palisade and in the whole mesophyll; in *Ilex argentina*, *Ilex dumosa* var. *dumosa* and *Ilex theezans* in the spongy parenchyma.

The following mean values are established as *stomata index* for each of the species and varieties:

Ilex paraguariensis var. *paraguariensis*: $\bar{X} = 10.13$

Ilex argentina: $\bar{X} = 12.38$

Ilex brevicuspis: $\bar{X} = 8.22$

Ilex dumosa var. *guaranina*: $\bar{X} = 10.93$

Ilex dumosa var. *dumosa*: $\bar{X} = 9.80$

Ilex theezans: $\bar{X} = 11.26$

As *palisade ratio* the following values:

Ilex paraguariensis var. *paraguariensis*: $\bar{X} = 3.00$

Ilex argentina: $\bar{X} = 7.95$

Ilex brevicuspis: $\bar{X} = 3.57$

Ilex dumosa var. *guaranina*: $\bar{X} = 2.31$

Ilex dumosa var. *dumosa*: $\bar{X} = 2.41$

Ilex theezans: $\bar{X} = 4.12$

According to the before exposed, it can be manifested the exo- and endomorphological characters accompanied by the numerical values: stomata index and palisade ratio, represent an original contribution and constitute a secure methodology of easy execution.

Ilex paraguariensis is used in the European and American market as raw material for obtaining the phytotherapeutic products, in different pharmaceutical forms, indicated as supplement dietary in hypocaloric regimens, as soon as diuretics and in the treatment of functional asthenia (Bruneton 1993; Rombi 1991).

Thus, the data presented in Table 1 are relevant since they summarize the characteristics studied, which identify each species and allow the establishment of genuinity, adulterations or substitutions.

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