

Micrographic Parameters of Primary Stem, Flower, Fruit, and Seed of *Terminalia australis*

María T. Castro, Stella M. Carpano, Javier Rossi, Marta T. Nájera, and Etilé D. Spegazzini

Laboratorio de Referencia de Análisis Micrográficos de Plantas Medicinales Alimenticias y Tóxicas (LABRAM)
Farmacobotánica, Depto. Cs. Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata,
La Plata, Argentina

Abstract

Terminalia australis Cambess (Combretaceae), known as *palo amarillo*, is an arboreal species, less frequently shrubby, and native of Brazil, Paraguay, Uruguay, and Argentina. Antimicrobial activity was verified through investigations carried out with aqueous extracts from leaves. The purpose of the current work is to study the exo-endomorphology of the aerial organs (primary stem, flower, fruit, and seed) of the mentioned species in order to establish micrographic parameters for a future pharmacopeic monograph. Fresh and dry materials were studied. Sections and macerations were obtained. Designs were obtained with light microscope equipped with a drawing tube, and photomicrographs were generated with scanning electron microscope. Numerical values were determined: lineal magnitude for the species that are presented together with the data typical of the histoanatomy of the aerial organs.

Keywords: Combretaceae, flower morphology, fruit morphology, micrography, primary stem morphology, seed morphology, *Terminalia australis*.

Introduction

Terminalia australis Cambess (Combretaceae), known as *palo amarillo*, has been described previously in Argentine papers on floristics (Exell, 1939; Cabrera & Dawson, 1944; Fabris, 1965; Leonardis, 1976; Lahitte & Hurrell, 1997a; Guaglianone, 1998, 1999), Brazil (Exell & Reitz, 1967), and Uruguay (Lombardo, 1964; Muñoz et al., 1993). The exo-endomorphology of the leaves has been studied by Castro et al. (2001) and its nervation pattern

by Klucking (1991). The pollen grains have been described with light microscope, on the basis of a specimen by Barth and Silva (1965) and Markgraf and D'Antoni (1978).

There are references dating back to the 19th century describing the use of this taxon bark as an astringent, due to the presence of tannins (Corrêa, 1984; Hieronymus, 1882). This information was confirmed by inhabitants of the Martín García Island (Lahitte & Hurrell, 1995). Also, it was mentioned (Bassols & Gurni, 1998) as a possible adulterant of *Lippia turbinata* Griseb. (Verbenaceae).

Investigations carried out with aqueous extracts from leaves demonstrated antimicrobial activity against *Staphylococcus aureus* (Escalante & Carpano, 1977). Later, Carpano and Carosanti (1998) demonstrated the bactericidal activity of aqueous extracts of leaves and stems against pathogenic strains of *Pseudomonas aeruginosa* isolated from human lesions. Recently, Carpano et al. (2003) verified the antifungal activity of aqueous and methanolic extracts of aerial organs against *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Candida albicans*, and *Candida kruszei*.

From the phytochemical point-of-view, Dominguez et al. (1928) obtained negative results concerning the presence of alkaloids, cyanoglucosides, saponins, oxidases, and peroxidases in foliaceous stems collected in December. Later, Hultin (1965) analyzed the leaves and root bark and detected alkaloids only in the former. The aim of the current work was to perform an exo-endomorphological study of the aerial organs (primary stem, flower, fruit, and seed) of the mentioned species, with the purpose of obtaining micrographical parameters for a future pharmacopeic monograph.

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Address correspondence to: María T. Castro, Calle 47 y 115, La Plata (1900), Argentina. Fax: 54-221-4254389;
E-mail: labram@biol.unlp.edu.ar

Materials and Methods

Fresh and dry materials of *T. australis* were analyzed. They were collected in Punta Lara, Province of Buenos Aires, and Arroyo Cuñapirú, Province of Misiones (Argentina), and in Real de San Carlos, Department of Colonia (Uruguay). These herborized samples have been incorporated into the Herbarium of the Museo de Botánica y Farmacognosia "Carlos Spegazzini" of the Facultad de Ciencias Exactas de La Plata, UNLP (LPE). The specimens come from the following Institutions: LP (Herbarium of Museo de La Plata, Argentina), LPA (Herbarium of Facultad de Agronomía, UNLP, La Plata, Argentina), MBM (Museo Botánico Municipal de Curitiba, Brazil), SI (Instituto Darwinion, San Isidro, Argentina), and UPCB (Herbarium of the Department de Botánica de la Universidad Federal de Paraná, Curitiba, Brazil).

Argentina

Province of Buenos Aires: Pta. Lara, Freire 3/11/79 (LPA), Punta Lara Natural Reserve, on the road to Villa Elisa 50 m from the coastal road, Castro 193 (LPE); Parador "El Tío," Castro 204 (LPE); 70 m from Boca Cerrada stream, Castro 197 (LPE); entrance to the Reserve, Castro 196 (LPE); next to the shed, Castro 234 (LPE). Arboretum Fac. Agronomía, Fabris 1882 (LP). San Isidro, Institute Darwinion Park, N.M. Bacigalupo s/nro. (SI 17462). Martín García Island, J. Hurrell et al. 1118, 1327, 3855 (LP), Gamero 1003 (LP). Province of Corrientes: Dpto. San Martín: R.N. 129 crossing above the Guavirarí stream, O. Boelcke, N.N. Correa, N. Bacigalupo et S.C. Arroyo nro. M.N. Correa 5347 (SI); La Cruz, A. Burkart 7871 hoja a (SI). Dpto. Santo Tomé: Krapovickas et al. 21083 (LP). Province of Entre Ríos: Dpto. Concepción del Uruguay: Concepción del Uruguay, Municipal Bathing Resort Itape, N.M. Tur, R. Guaglianone et C.N. Skorupka 1908 (SI). Dpto. Concordia: A.L. Cabrera et Sagastegui 19304 (LP); N. Berti et Escalante 272 (LP). Cambacua Island, A.L. Cabrera 12330 (LP). Delta, Brazo largo A.C. Scala s/nro. (LP 38366). Province of Misiones: Dpto. Cainguas: UNLP Private Reserve "Valle del Arroyo Cuñapirú," on the banks of Cuñapirú stream, Castro 231 (LPE), 3/20/01. Dpto. Candelaria: Santa Ana, Rodríguez 602 hoja a (SI); Dpto. Concepción, A.L. Cabrera et al. 28679 (SI). Dpto. San Ignacio: Salto Tabay, O. Boelcke, N. Correa et al. 5382 hojas a y b (SI). Dpto. Montecarlo: Pto. Piray, S.A. Renvoize 3193 (MBM). Dpto. San Pedro: Río Pitay-Guazú, O. Boelcke et al. 5487 (SI).

Brasil

Est Paraná: Mun. Curitiba: Loc. Encas, G. Hatschbach 35171 (LP). Mun. Foz do Iguaçu: Itaipú, J.A. Cordoni

s/nro. (UPCB 21954). Mun. Três Barras do Paraná: Rio Guaraní, S.M. Silva s/nro. (UPCB 20681), I. Isernhagen et M. Borgo 123 (UPCB). Mun. Vizin- hos: Foz do Chopim, G. Hatschbach 20575 (LP, SI, UPCB 7993). Est. Santa Catarina: Mun. Itapir- anga: 3–4 km West from Itapiranga, L.B. Smith et Klein 13169 (LP). Mun. Riqueza: Rio Iracema, L.B. Smith et Klein 13117 (LP, SI). Mun. São Miguel d'Oeste: Peperi, L.B. Smith et Klein 13246 (SI).

Paraguay

Dpto Itapúa: Alto Paraná, Encarnación, T. Rojas 10824 (SI).

Uruguay

Dpto. Colonia: Colonia del Sacramento, coastal road to Real de San Carlos, Castro 194 (LPE); Real de San Carlos, opposite the Municipal Camping Site, Castro 195 (LPE); Riachuelo, A.L. Cabrera 3905 (LP); Playa Ferrando, Castro 233 (LPE). Dpto. Río Negro: Loc. San Javier, G. Herter 11/27, ex- herb 82822 (SI).

Cuts of primary stem, flower, fruit, and seed (transverse, longitudinal, radial, and tangential, as it corresponds) and primary stem and fruit macerations with 10% NaOH and Jeffrey mixtures were obtained; diagnostic histochemical reactions were performed to show calcium oxalate (hydrochloric acid), tannins (ferric chlorate, potassium iodate), alkaloids (Dragendorff), aleurone (xylene, picric acid), starch (solution of iodine), lipids (Sudan III), and lignin (phloroglucinol solution).

In reference to the technique used for the observation of the primary stem, anther, fruit, and seed with a scanning electron microscope (SEM), the fresh material was washed with chlorinated distilled water and fixed in FAA for 48 h. With respect to the grains of nonaceto- lyzed pollen, they were washed with absolute alcohol. Later, the materials were mounted and dried on an aluminum plate. The observations and photomicro- graphs were performed with a Jeol JSM-T100 from the Museum de ciencias Naturales SEM Service in La Plata.

According to Erdtman (1969), acetolyzed grains, mounted in glycerine-gelatin and sealed with paraffin, were observed under a light microscope (LM). Prepara- tions of nonchemically treated pollen grains were made with Wodehouse's technique (1935) in order to compare them with the acetolyzed pollen. The shape was defined in accordance with the Dp/De relation (Erdtman, 1952) and in measuring the pores, the polar axis being indicated first and then followed by the equatorial axis. The palynological terminology used here agrees with Punt et al. (1994). All the values obtained from each

parameter were expressed in ranges, average, mode (Mo), and standard deviation(s).

Observations were made in Farmacobotánica, Facultad de Ciencias Exactas, with a stereoscopic microscope Iroscope YZ-6 and a light microscope Olympus CH, and the original designs were made with this microscope equipped with a drawing tube Iroscope. The symbols used in the designs correspond to those of Metcalfe and Chalk (1950).

Results

Terminalia L., Mant., 1:21, 1767. (Of the Latin *terminus*: end, apex referring to the leaves grouped in the twig apices. *Terminalia australis* Cambessedes, in Saint-Hilaire, Fl. Bras. Merid., 2:240, t. 128, 1829.)

Synonymy: *Myrobalanus australis* (Cambess.) Kuntze, Rev. Gene. 1:237, 1891.

Material type: The material that documents *Terminalia australis*, described by Cambessedes, comes from: “Ad ripas rivuli vulgo Toropasso ad amnem Ibicuy in provinciâ Missionum; necnon in sylvis prope prædium vulgo S. Juan haud longè ab urbe Colonia do Sacramento.” Later, Eichler (1867:85) quotes: “In Brasilia austro-orientali: Sello; ad ripas rivuli vulgo Toropasso ad amnem Ibicuy in prov. Paraná, nec non in silvis prope prædium vulgo S. Juan haud longè ab urbe Colonia do Sacramento: St. Hilaire.”

Vulgar names: In Spanish, *palo amarillo*, *amarillo*, *amarillo del río*, *blanquillo*, *tanimbú*; in Guarani, *ihvhrá saithdyú*; in Portuguese, *amarilho*, *sarandi amarelo*, *amarelinho*; in English, *bombawy* (Bertoni 1994 Corrêa, 1984; Lahitte & Hurrell, 1997b; Backes & Nardino, 1999). The vulgar names are due to the ochre-yellow color of the wood. The following taxa are also designated under the vernacular name of *palo amarillo*: *Terminalia triflora* (Griseb.) Lillo (Combretaceae), *Berberis buxifolia* Lam. (Berberidaceae), *Phyllostylon rhamnoides* (Pois.) Taub. (Ulmaceae), and *Aloysia gratissima* (Gillies et Hook.) Troncoso (Verbenaceae).

Vegetative description of the species: Tree, 4–15 m high and 40-cm diameter, generally with stretched trunks, branched out from the base, less frequently shrubby. The outer corky bark occupies 20% of the total bark, is covered with crustacean lichen, and has shallow fissures of irregular type, of approximately parallel arrangement, with rounded or flattened ridges; manner in which the scaly bark comes off; adhered scales, with irregular form. The inner bark occupies 80% of the total bark, has fibrous texture and corrugated pattern and is wet to the tact. Branches are puberulous when young and glabrous when adult. Simple leaves with sides in different colors are alternate or in pseudo-verticil, narrowly oval or

oblanceolate, 4–6 times longer than broad, 2–12 cm long, generally in acute apex, brochidodromous nervation, with 4–7 couples of lateral veins shown in the abaxial face, shortly peciolate.

Fenology: It blooms in spring and fructifies in summer and autumn.

Geographical distribution: It is a hygrophyte species that grows in southern Brazil, Paraguay, Uruguay, and northeastern Argentina, up to the Parana delta, La Plata shore, and Martín García Island. Altitudinally, it grows from 0 to 500 m above sea level (Guaglianone, 1999).

Ecological observations: The specimens observed in Misiones, on the banks of Cuña Pirú stream and Piray Guazú stream (Argentina), present dark green leaves, without reddish coloring, and more grayish stems than the ones found in Buenos Aires, Punta Lara. This is probably due to the greater quantity of arboreal strata that limit the amount of sunlight reaching the lowest strata; the possibility that edaphic factors contribute to those features has not been discarded.

Exell and Reitz (1967) argue that these specimens protect the banks of the rivers from erosion.

Structural analysis of the primary stem

The epidermis is unistratified, with straight cellular walls, abundant “combretaceous” trichomes; anomocytic stomata are scarcely frequent (Fig. 1C). The primary cortex is made of an angular collenchyma (Fig. 1E) with crystaliferous prismatic and enlarged idioblasts, containing calcium oxalate druses of 16.50 (27.00) 43.30 μm , Mo = 26.60 μm , s = 2.13 (Figs. 1A and 2A). The most internal layer of the cortex has an endodermoidic nature (Fig. 2C). The vascular tissues form a eustele of bicollateral bundles. The pericycle contains isolated strands of sclerenchymatic fibers placed around a large inner parenchyma portion (Figs. 1A and 2C). The internal phloem is weakly developed (Fig. 1A). The external phloem has crystaliferous idioblasts arranged in tangential rows, with calcium oxalate druses of 3.30 (6.70) 10.00 μm , Mo = 6.70 μm , s = 0.60 (Fig. 1A). Elements of vessel with annular, spiral, scalariform, and pitted thickening; with oblique to very oblique partitions, of 230.00 (320.00) 515.00 μm , Mo = 233.00 μm , 333.00 μm , s = 20.48 long, with appendices of up to 50.00 μm . The vessels are not isolated as in the old stem but arranged in groups of radial rows. The intervacular bordered pits are small, rounded to oval, vestured and alternate. Axial parenchyma have simple, lengthened prismatic crystals of calcium oxalate of 16.60 (27.70) 40.00 μm , Mo = 23.30 μm , s = 1.90. Xylematic fibers with simple pits, 400.00 (695.00) 975.00 μm , Mo = 880.00 μm , s = 13.34 long. The pith is formed by angular collenchyma

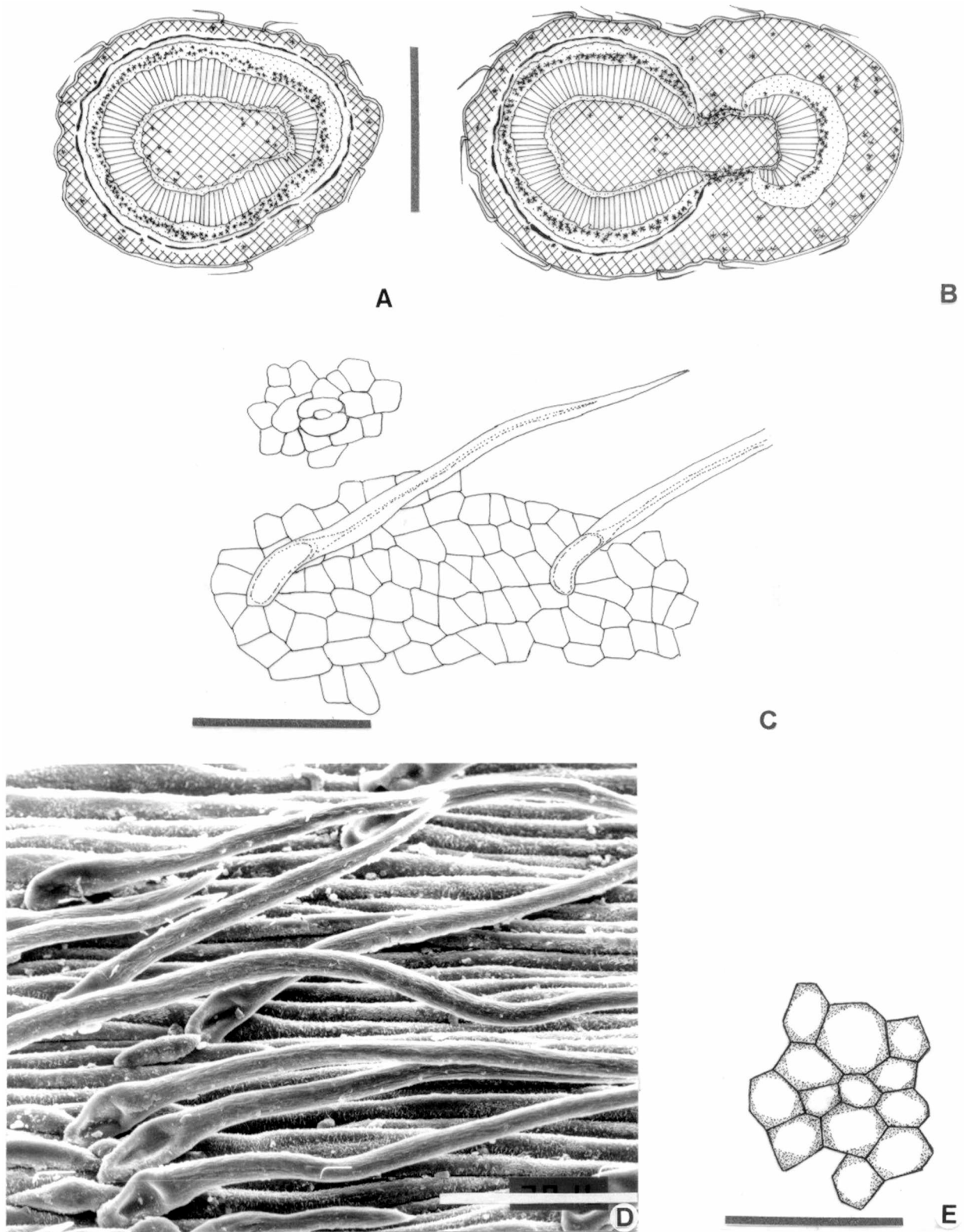


Figure 1. Primary stem. (A) Primary stem; (B) relation between the vascular system of the stem and the leaf; (C) epidermis in superficial view; (D) SEM photomicrography of trichomes; (E) collenchyma in transverse cut. Bar size: 100 μm (C–E); 1 mm (A, B).

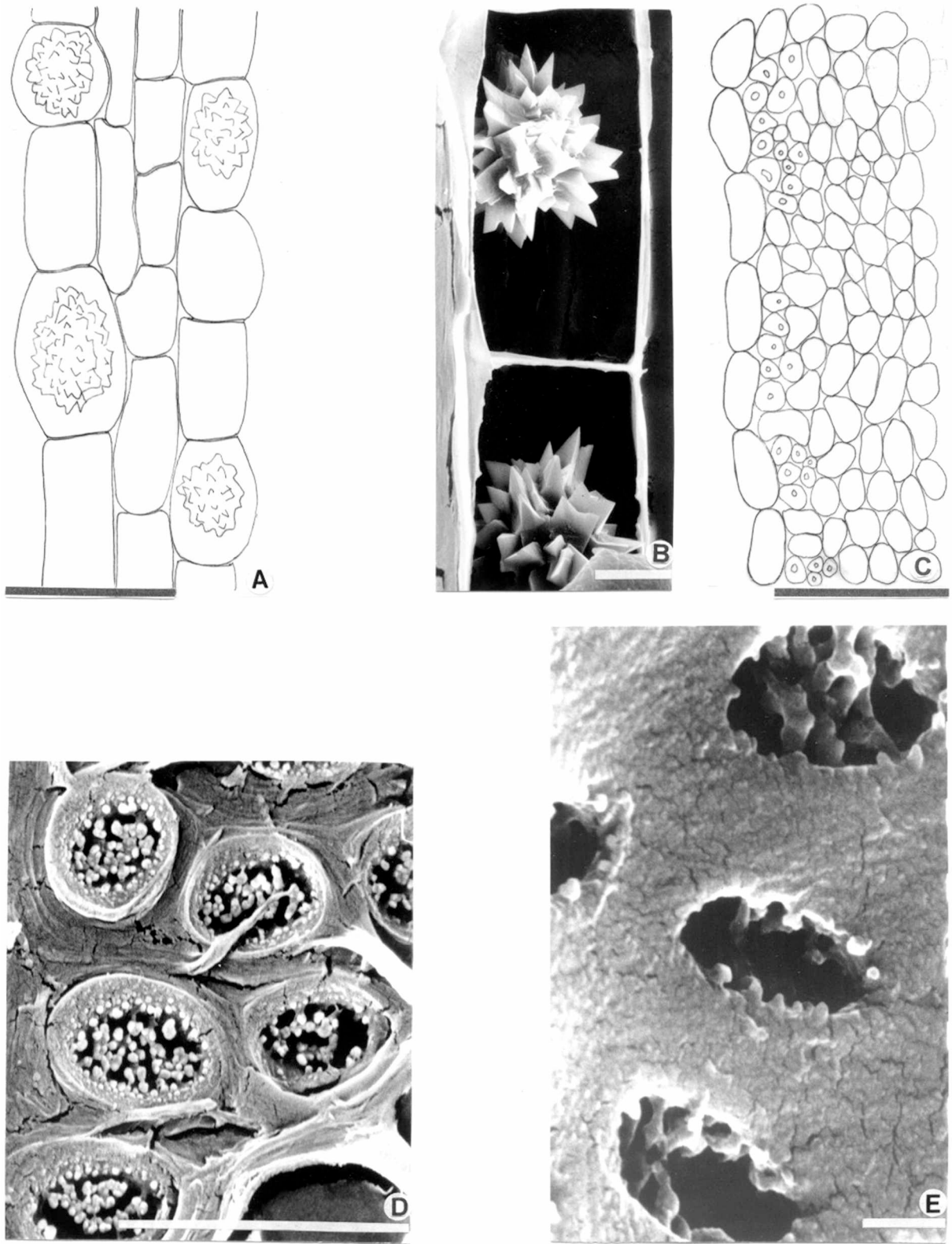


Figure 2. Primary stem. (A) crystaliferous idioblasts of cortex in longitudinal cut; (B) SEM photomicrograph of crystaliferous idioblasts of cortex; (C) endodermoid layer, fibers, and pericyclic parenchyma in transverse cut; (D, E) SEM photomicrograph, details of external and internal view of the vestured pits, respectively. Bar size: 1 μm (E); 10 μm (B, D); 100 μm (A, C).

with crystaliferous idioblasts containing calcium oxalate druses of 10.00 (19.80) 33.30 μm , $\text{Mo} = 20.00 \mu\text{m}$, $s = 1.43$ (Fig. 1A).

Unilacunar node (Fig. 1B).

SEM: The cuticle of the trichomes is formed by parallel striae (Fig. 1D). Vestured pits as thick trunks with branches, or barely branched out, toward the interior of the areolar cavity (Figs. 2D and 2E).

Histochemistry: Positive for tannins and alkaloids.

Structural analysis of flower

Inflorescences in subcapitate axillary spikes have slighty pilose peduncle 2–4.5 cm long. Three to 12 perfect yellowish flowers, on a rachis 1–12 mm long; pilose bracts 2–3 mm long. Flowers are 6–9.2 mm; the hipanthium is divided into a tubuliform, densely sericeous, lower part 2–2.5 mm long, adnate to the ovary and a cupuliform, pubescent upper part 2–2.5 mm long (Fig. 3A). Sepals 5, triangular–egg-shaped, reflexed, of about 1 mm, pubescent, valvate. Absence of petals. Stamens 10, biseriate, first cycle antisepals and second cycle alternisepals, exerted, versatile anthers, longitudinal dehiscence. Epigynous lobate glandular disk, 2–2.5 mm in diameter, adnate to the upper hipanthium. Unilocular ovary. Anatroous, pendulous ovules. Exserted, cylindrical style, of about 4.5 mm, simple stigma.

Hipanthium

Lower hipanthium external epidermis is formed by cells of straight to slightly wavy walls, with anomocytic stomata and “combretaceous” trichomes (Fig. 3B) that contain crystaliferous idioblasts with calcium oxalate druses of 10.00 (17.00) 27.00 μm , $\text{Mo} = 16.60 \mu\text{m}$, $s = 1.18$ among the vascular bundles. The external epidermis of the upper hipanthium is formed by cells of straight to slightly wavy walls, with anomocytic stomata and “combretaceous” trichomes (Fig. 3C). The internal epidermis of the upper hipanthium is formed by cells of straight to slightly wavy walls, with “combretaceous” trichomes (Fig. 3D). Between both epidermis there is one parenchyma with some intercellular spaces, in the layers near the internal epidermis, while there are crystaliferous idioblasts with calcium oxalate druses of 6.50 (11.60) 30.00 μm , $\text{Mo} = 10.00 \mu\text{m}$, $s = 1.36$ (Fig. 3E).

Glandular disk

The external epidermis is formed by cells of straight to slightly wavy walls, with anomocytic stomata and very scanty “combretaceous” trichomes. Among secretory cells, there are crystaliferous idioblasts with calcium oxalate druses of 20.00 (20.80) 26.65 μm , $\text{Mo} = 20.00 \mu\text{m}$, $s = 1.03$.

Sepals

The external epidermis has straight to wavy walls with anomocytic stomata (Fig. 3F). The internal epidermis is formed by cells of straight to wavy walls (Fig. 3G); both have “combretaceous” trichomes (Figs. 3F and 3G). Between both there is one parenchyma with some intercellular spaces, in the layers near the internal epidermis, there are crystaliferous idioblasts with calcium oxalate druses of 6.50 (13.50) 23.50 μm , $\text{Mo} = 13.00 \mu\text{m}$, $s = 1.55$.

Gynoecium

The style epidermis has a prosenchymatous aspect, cells with striated cuticle, the basal third with “combretaceous” trichomes and the rest glabrous (Fig. 3H). The parenchyma has crystaliferous idioblasts with calcium oxalate druses of 6.50 (14.00) 30.00 μm , $\text{Mo} = 16.65 \mu\text{m}$, $s = 2.02$. The ovary has crystaliferous idioblasts with calcium oxalate druses of 3.30 (6.40) 10.00 μm , $\text{Mo} = 6.50 \mu\text{m}$, $s = 0.64$. Ovules 2, crasinucellate, bitegmic, with long funicle.

Stamens

Filament: the epidermis has rectangular cells with a striated cuticle (Fig. 3J) and surrounds an angular collenchyma that contains a vascular bundle, elements of vessel with annular and spiral thickenings.

Anther: epidermis with striated cuticle, with a conspicuous endothecium outstanding to the maturity, with lignin thickenings, glandular tapetum, and 4 pollen sacs. Crystaliferous idioblasts with calcium oxalate druses under the epidermis of 5.00 (8.00) 10.00 μm , $\text{Mo} = 6.50 \mu\text{m}$, $s = 0.50$ and among the pollen sacs of every thecae of 13.30 (16.00) 23.30 μm , $\text{Mo} = 16.60 \mu\text{m}$, $s = 0.84$.

SEM: Cuticle of the anther with numerous tortuous striae (Fig. 3K). Thickened band-shaped endothecium (Fig. 4A).

Pollen

Radially symmetric isopolar grains; subprolate to spheroidal; amb lobate (Fig. 4D); heterocolpate, with 3 colporus and 3 pseudocolpus; colpus and pseudocolpus near the poles; circular to lalongate os, sometimes protrusives. Tectate exine. Dimensions: polar diameter 13.60 (17.00) 20.50 μm , $\text{Mo} = 16.30 \mu\text{m}$, $s = 0.88$; equatorial diameter 11.00 (14.00) 16.30 μm , $\text{Mo} = 15.00 \mu\text{m}$, $s = 0.90$; diameter of os 1.90 (2.50) 3.00 μm , $\text{Mo} = 2.70 \mu\text{m}$, $s = 0.21 \times 4.00$ (4.60) 5.50 μm , $\text{Mo} = 4.00 \mu\text{m}$, $s = 0.46$; thickness of the exine in the poles 1.4 μm .

SEM: surface with punctas and nanorugulate (Figs. 4C, 4E, and 4F).

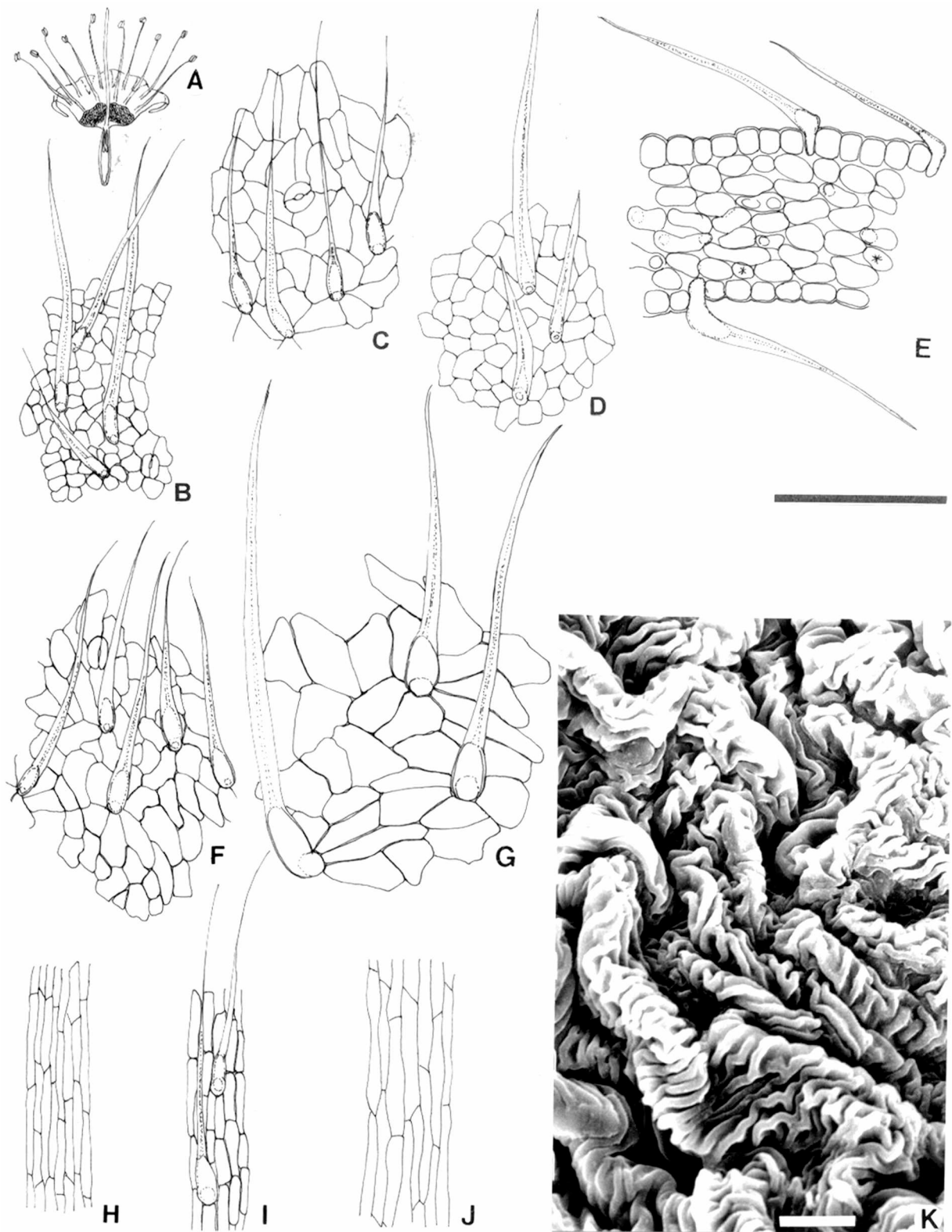


Figure 3. Flower. (A) Hipanthium partially unfolded; (B) superficial view of the external epidermis of the lower hipanthium; (C, D, E) superficial view of the external and internal epidermis and transverse cut of the upper hipanthium, respectively; (F, G) superficial view of the external and internal epidermis of the sepals, respectively; (H, I) superficial view of the middle and basal sector of the epidermis of the style, respectively; (J) superficial view of the epidermis of the staminal filament; (K) SEM photomicrography detail of cuticle of anther. Bar size: 10 μm (K); 100 μm (B–J).

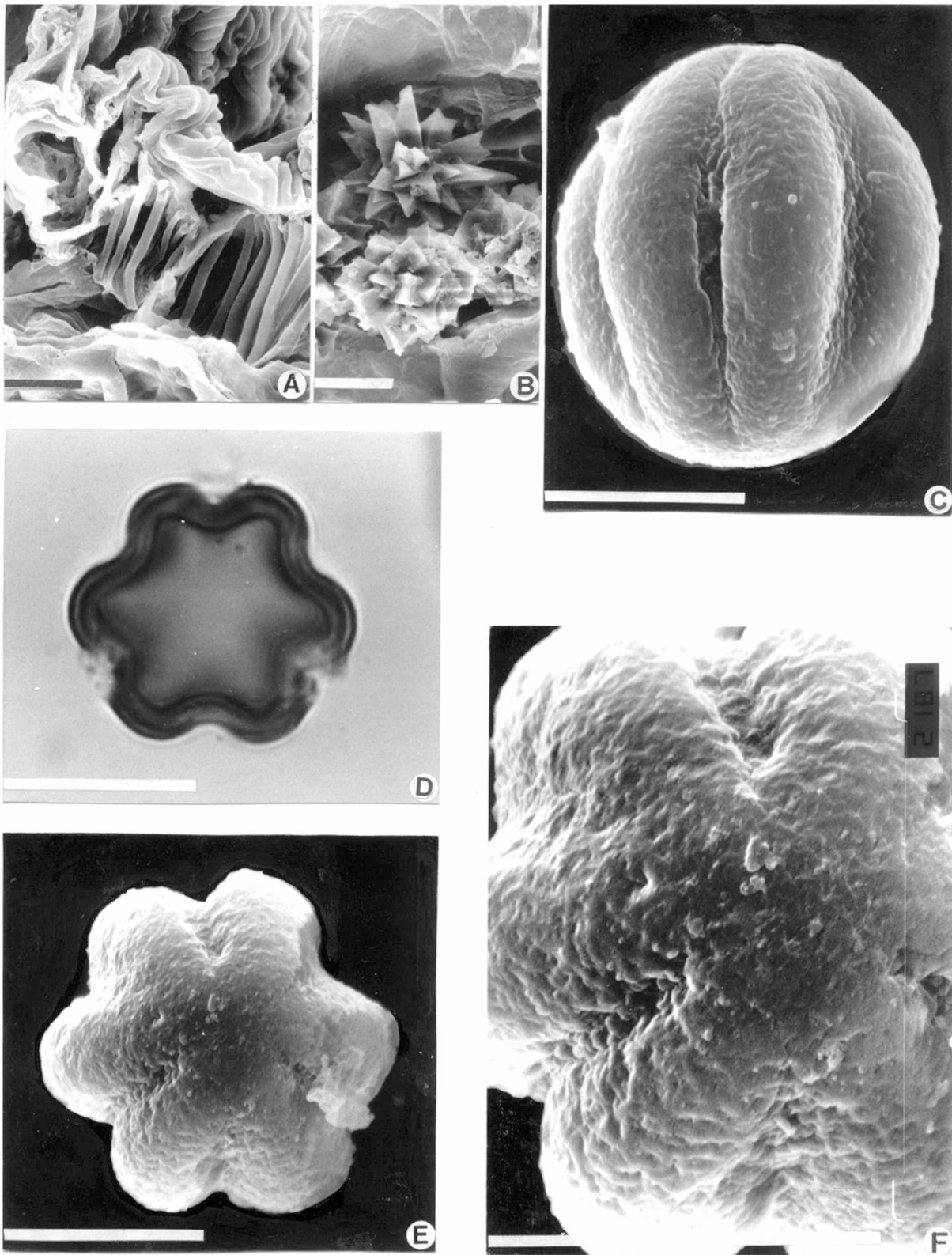


Figure 4. Flower (A, B, C, E, F) SEM photomicrography, (A) thickening of endothecium, (B) druses between the pollen sacs, (C) equatorial view of pollen, (E) polar view, (F) detail of the sculpture, (D) LM photomicrography of pollen in polar view. Bar size: 10 μm (A–F).

Histochemistry: Positive tannins in hipanthium, glandular disk, sepals, stamens, and ginoecium.

Structural analysis of the fruit

Pendulum, ellipsoidal or egg-shaped woody fruit approximately 1.7–2.5 cm long by 0.7–1.7 cm wide; one of the faces is convex with a more or less marked rib; the opposite face with a longitudinal little marked rut, pointed; narrow marginal wing 1–4 mm wide, indehiscent.

Pericarp: The epicarp is made up of one layer of cells, of polygonal contour in superficial view, with “combretaceous” trichomes and anomocytic stomata (Fig. 5C). The mesocarp is formed by approximately six parenchymatic-cell layers (Figs. 5A, 5B, and 5G). The external endocarp is made up of sclerenchymatic fibers of 221.00 (508.00) 860.00, Mo = 312.00, 600.00 μm , $s = 12.67$ long (Figs. 5A and 6A). The middle endocarp is formed by sclereids (brachysclereids, astrosclereids, and macro-sclereids), sclerenchymatic fibers of 203.00 (283.00) 366.00 μm , Mo = 283.00 μm , $s = 15.53$ long, with crystaliferous idioblasts that contain calcium oxalate druses 26.60 (34.00) 43.00 μm , Mo = 36.60, $s = 1.57$, and 5 vascular bundles with one external cap of sclerenchyma (Figs. 5A and 6A). The internal endocarp is made up of sclerenchymatic fibers of 208.00 (400.00) 550.00 μm , Mo = 170.00 μm , $s = 6.84$ long and fibers with calcium oxalate prismatic crystals of 13.30 (19.00) 30.00 μm , Mo = 16.60 μm , $s = 1.19$ long (Figs. 5A and 6A).

SEM: The trichome cuticle presents parallel striae (Figs. 5D and 5E). The epicarp is covered with an amorphous wax film upon which semicrystalline and crystalline structures are found superimposed in the form of crusts and plates (Fig. 5F). Sclereids and sclerenchymatic fibers of the endocarp with simple pits of 0.22–2.30 μm (Fig. 6B) and 0.50–3.00 μm in diameter, respectively (Fig. 6C). Polyhedral crystaliferous idioblasts of the endocarp (Fig. 6D).

Peduncle

The epidermis has cells of straight walls, with “combretaceous” trichomes (Fig. 7F). The primary cortex is made up of an angular collenchyma with crystaliferous idioblasts containing calcium oxalate druses of 20.00 (28.50) 40.00 μm , Mo = 26.50 μm , $s = 1.44$. The most internal layer of the primary cortex has an endodermoidic nature. Eustele of bicollateral bundles. Pericycle made up of a collenchymatic sheath that is sometimes lignified and an inner portion of parenchyma. The internal phloem is weakly developed (Fig. 7E). The external phloem has crystaliferous idioblasts that contain calcium oxalate druses of 3.30 (5.00) 6.70 μm , Mo = 5.00 μm , $s = 0.37$. The pith is formed by an angular collenchyma with crystaliferous idioblasts that contain calcium oxa-

late druses of 13.30 (19.00) 23.30 μm , Mo = 20.00 μm , $s = 0.60$ (Fig. 8E).

Histochemistry: Positive tannins in immature and mature fruits (pericarp and peduncle).

Structural analysis of the seed

The spindle-shaped naked seed is 5–12 mm long by 2 mm wide, without wing; the seed-coat is light-chestnut colored, with an apical and roundish chestnut-colored raphe.

A dull pale yellow exotesta is formed by cells of straight-to-briefly-wavy cellulose walls, without stomata (Fig. 8C). The yellowish-chestnut colored mesotesta is made up of sclerotic cells (Fig. 8D). Yellowish-chestnut colored endotesta and exotegmen are made up of tracheidal cells and have spiral thickening (Fig. 8F). The pale yellow endotegmen is formed by 2–3 layers of cells of thin, tangentially lengthened and flattened walls (Fig. 7A).

Non-endospermic; the bulky fleshy cotyledons fill the seminal cavity, of convolute veneration (Fig. 8B), cells with reserve substances and calcium oxalate druses of 23.30 (37.00) 47.00 μm , Mo = 36.60 μm , $s = 2.09$ (Figs. 8B and 7B).

SEM: At the raphe level, the exotesta is made up of narrower cells compared to the surrounding ones (Fig. 8A). The sclerotic cells of the mesotesta have roundish to lineal simple pits (Fig. 8E). The endotesta has a spiral thickening of 0.80–1.00 μm (Figs. 8E and 7G). Cotyledon globular aleurone grains of mainly granular surface of 0.66–1.33 μm (Fig. 8C).

Histochemistry: Positive tannins in seed-coats. Lipids (drops of oil) in the exotesta and cotyledons. Aleurone (with 1–2 globoids and 1–3 crystalloids) in cotyledons (Fig. 6C). Very scanty secondary starch in cotyledons.

Discussion and Conclusions

The primary stem of *T. australis* with bicollateral vascular bundles, the pericycle formed by fibers of sclerenchyma and parenchyma, and the presence of druses seem to be the features shared by members of the Combretaceae according to the descriptions of Metcalfe and Chalk (1950) and Verhoeven and Van Schijff (1975).

In 37 *Terminalia* species, Vliet (1979) has established type-B vestured pits with the forms 2, 3, and intermediate between both. The analysis with SEM of *T. australis* pits shows that they are intermediate between the forms 2 and 3. Vliet (1979) has considered that diagnostic value of this feature is doubtful, except for the fact that it is the only genus of Combretaceae that presents such variation. The fruit of *T. australis* is woody and dry, of samaroid type, and not a drupe as Corrêa (1984) states.

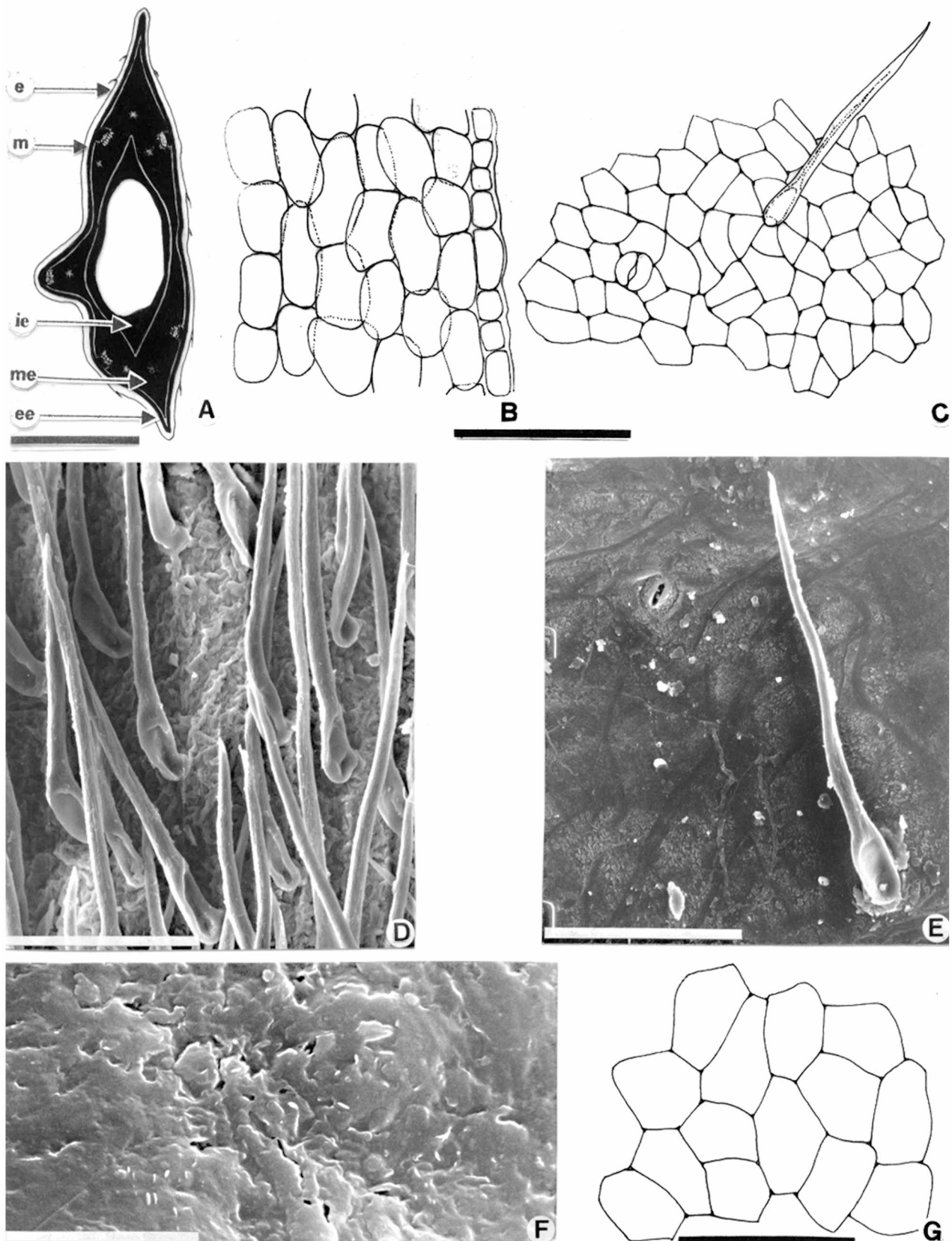


Figure 5. Fruit. (A) Fruit in transverse cut; (B) epicarp and mesocarp in transverse cut; (C) epicarp in superficial view; (D–F) photomicrographies SEM of the immature and mature epicarp and epicuticular wax details respectively; (G) first layer of the mesocarp in superficial view. Bar size: 10 μm (F); 100 μm (B–E, G); 5 mm (A).

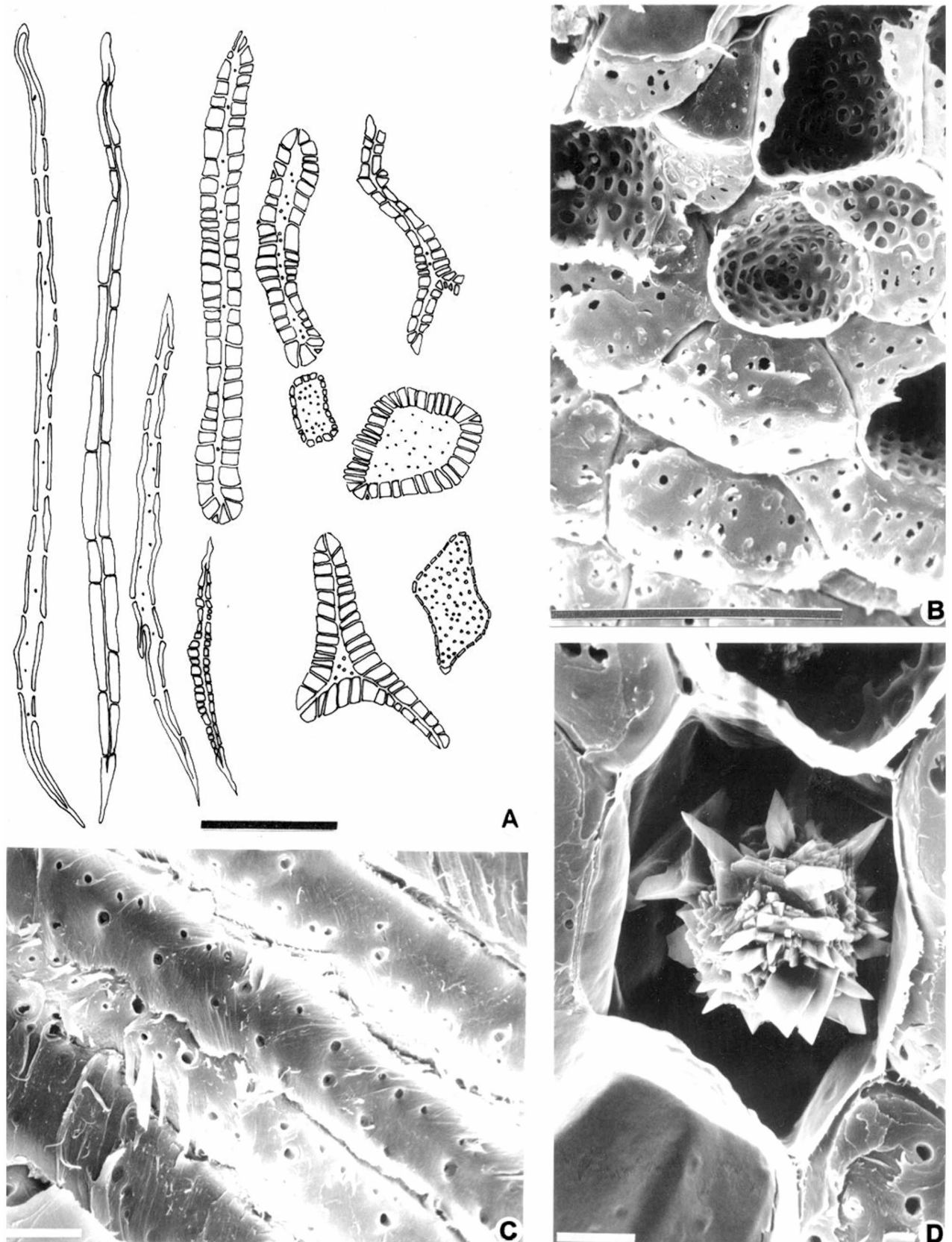


Figure 6. Fruit. (A): Sclerenchymatic and sclereid fibers of the endocarp; (B–D). SEM photomicrographies, (B) sclereids of the endocarp, (C) sclerenchymatic fibers of the endocarp, (D) crystaliferous idioblasts of the endocarp. e, epicarp; m, mesocarp; ee, external endocarp; me, middle endocarp; ie, internal endocarp. Bar size: 10 μ m (C, D); 100 μ m (A, B).

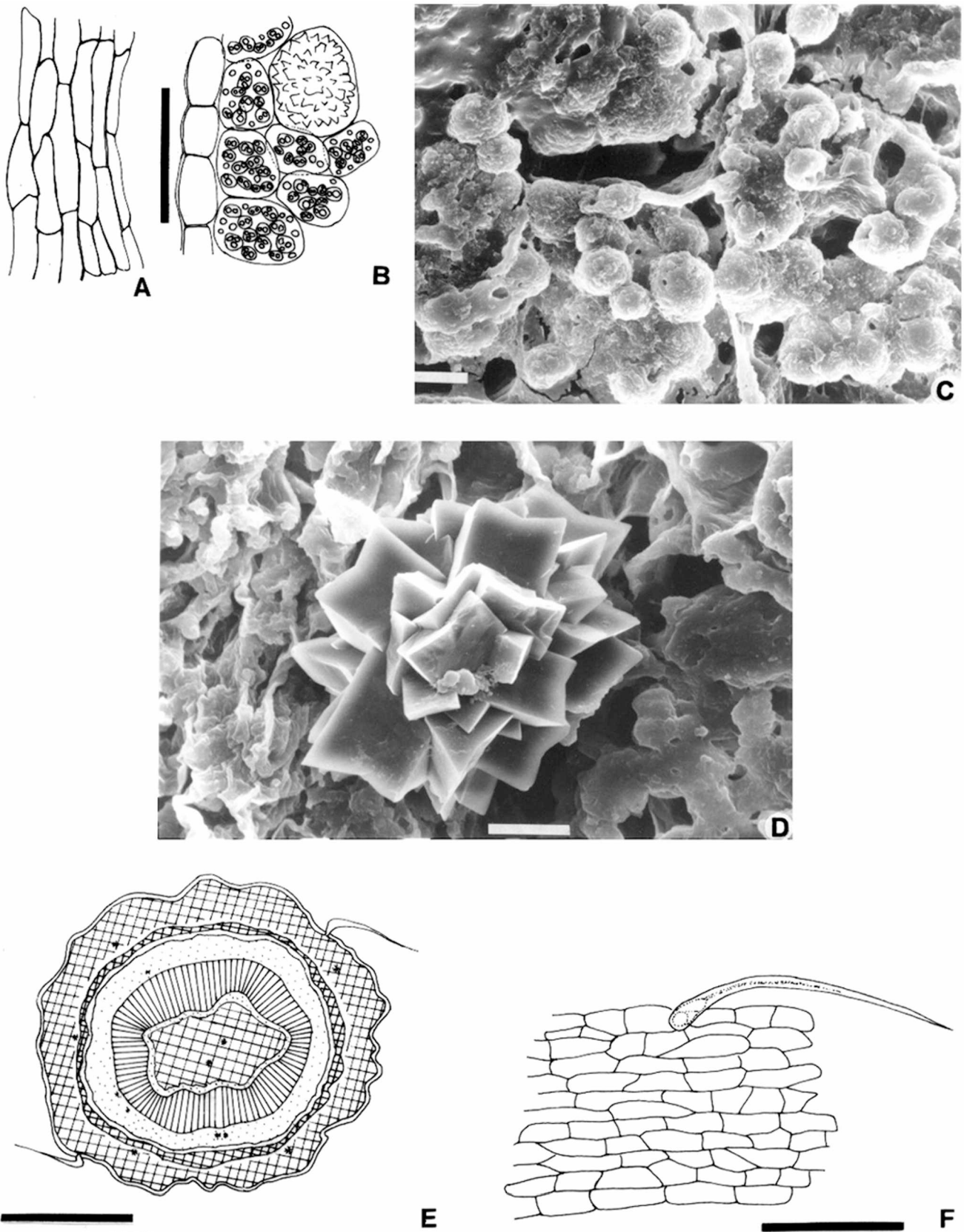


Figure 7. Seed. (A) Endotegmen in superficial view; (B) cotyledons in transverse cut; (C, D) SEM photomicrography, (C) aleurone of the cotyledons, (D) druse in the cotyledons. Peduncle of the fruit. (E) transverse cut; (F) superficial view of the epidermis. Bar size: 10 μm (C, D); 50 μm (A, B); 100 μm (F); 500 μm (E).

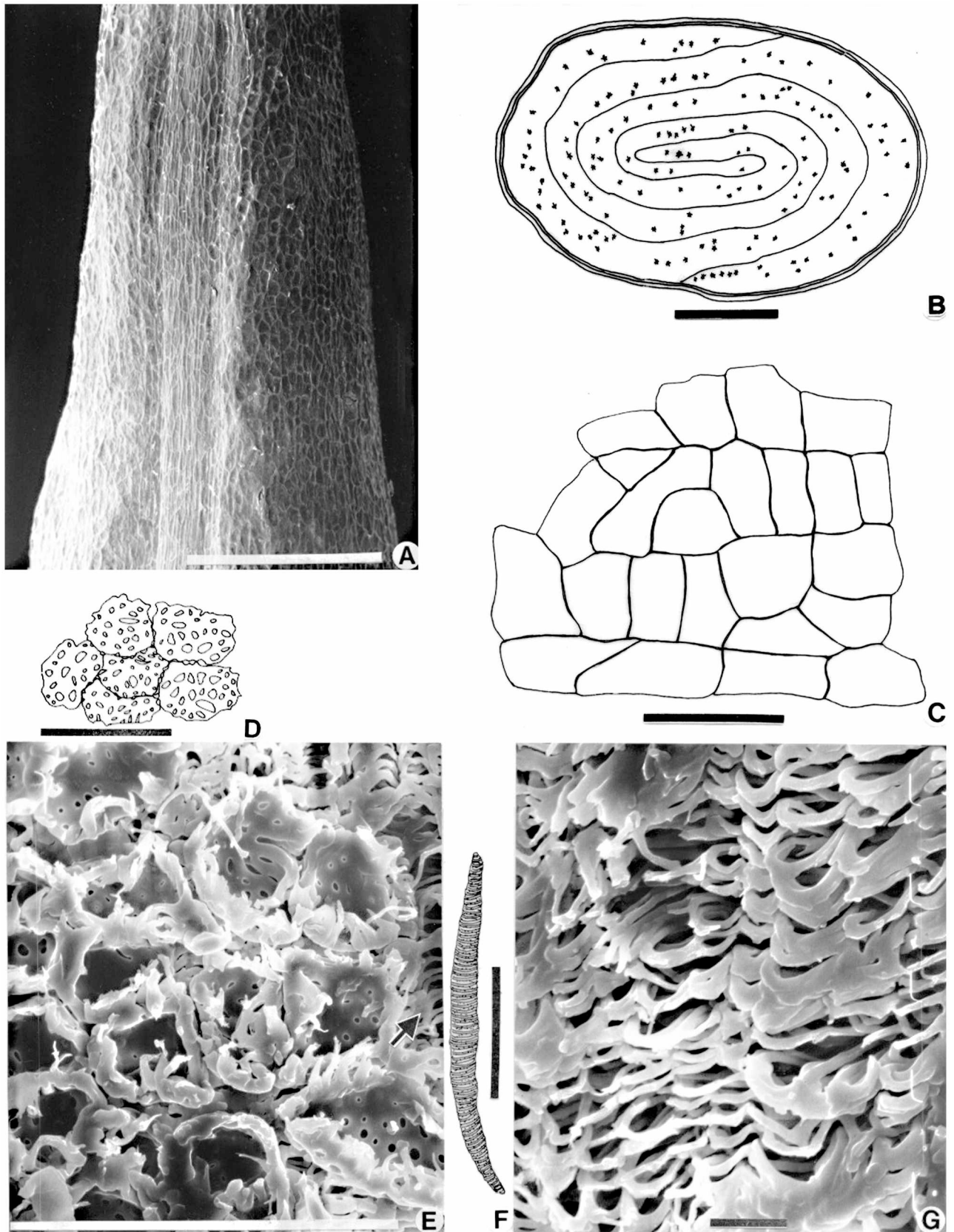


Figure 8. Seed. (A) SEM photomicrography of a sector of the seed with central raphe; (B) seed in transverse cut with cotyledons of convolute vernation; (C) exotesta in superficial view; (D) sclerotic cells of mesotesta in superficial view; (E) SEM photomicrography of sclerotic cells of mesotesta in tangential cut and tracheidal cells of the endotesta (arrow); (F) tracheidal cell of the endotesta; (G). SEM photomicrography of tracheidal cells of the endotesta. Bar size: 10 μm (G); 50 μm (D, F); 100 μm (B, C, E); 1000 μm (A).

Table 1. Quantitative micrographic parameters of primary stem for the identification of *Terminalia australis*.

Primary stem	Druses	Cortex	16.50 (27.00) 43.30 μm , Mo = 26.60 μm
		Phloem	3.30 (6.70) 10.00 μm , Mo = 6.70 μm
		Pith	10.00 (19.80) 33.30 μm , Mo = 20.00 μm
	Simple crystals	16.60 (27.70) 40.00 μm , Mo = 23.30 μm	
	Xylem	Elements of vessel	230.00 (320.00) 515.00 μm , Mo = 233.00, 333.00 μm
Fiber		400.00 (695.00) 975.00 μm , Mo = 880.00 μm	

Table 2. Quantitative micrographic parameters of flower for the identification of *Terminalia australis*.

Flower	Druses	Hipanthium	6.50 (13.60) 30.00 μm , Mo = 10.00 μm	
		Glandular disk	20.00 (20.80) 26.65 μm , Mo = 20.00 μm	
		Gynoecium	Style 6.50 (14.00) 30.00 μm , Mo = 16.65 μm ; Ovary 3.30 (6.40) 10.00 μm , Mo = 6.50 μm	
	Sepals	Sepals	6.50 (13.50) 23.50 μm , Mo = 13.00 μm	
		Anther	Subepidermics	5.00 (8.00) 10.00 μm , MO = 6.50 μm ,
			Between pollen sacs	13.30 (16.00) 23.30 μm , MO = 16.60 μm
	Pollen	Diameters	Polar	13.60 (17.00) 20.50 μm , Mo = 16.30 μm ,
			Equatorial	11.00 (14.00) 16.30 μm , Mo = 15.00 μm
			Ora	1.90 (2.50) 3.00 μm , Mo = 2.70 μm \times 4.00 (4.60) 5.50 μm , Mo = 4.00 μm
		Thickness exine	1.40 μm	
	Number of ovules	2		
	Number of teguments	2		

Table 3. Quantitative micrographic parameters of fruit for the identification of *Terminalia australis*.

Fruit	Druses	Endocarp	26.60 (34.00) 43.00 μm , Mo = 36.60
		Peduncle	Cortex 20.00 (28.5) 40.00 μm , Mo = 26.50 μm Phloem 3.30 (5.00) 6.70 μm , Mo = 5.00 μm Pith 13.30 (19.00) 23.30 μm , Mo = 20.00 μm
	Simple crystals		13.30 (19.00) 30.00 μm , Mo = 16.60 μm
	Length fibers of endocarp		203.00 (397.00) 860.00 μm , Mo = 312.00, 468.00 μm
	Number vascular bundles		5

Table 4. Quantitative micrographic parameters of seed for the identification of *Terminalia australis*.

Seed	Druses	Cotyledons	23.30 (37.00) 47.00 μm , Mo = 36.60 μm
	Number of layers	Exotesta	1
	Coat-seed	Mesotesta	1
		Endotesta	1
		Exotegmen	1
	Endotegmen	2–3	

The qualitative features of the aerial organs that have diagnostic value in the contribution to characterize the drug, with LM, are as follows.

Stem

Primary structure

- “Combretaceous” trichomes in the epidermis.
- Anomocytic stomata in the epidermis.
- Calcium oxalate druses in primary cortex, external phloem (in tangential rows), and pith.
- Vascular structure: eustele of bicollateral bundles.
- Pericycle made up of strands of sclerenchymatic fibers and parenchyma.
- Elements of vessel with plate of simple perforation and annular, spiral, scalariform, and pitted thickening.
- Elements of vessel with vestured and alternate pitting.
- Axial parenchyma with simple prismatic calcium oxalate crystals.
- Tannins and alkaloids.

Flower

- “Combretaceous” trichomes in the external face of the lower hipanthium, both faces of upper hipanthium, both faces of sepals and the basal third of the style, and very scanty trichomes in the glandular disk.
- Anomocytic stomata in the external epidermis of hipanthium, external epidermis of sepals, and glandular disk.
- Calcium oxalate druses in the anthers, gynoecium, hipanthium, sepals, and glandular disk.
- Crasinucellate ovules with long funicle.
- Isopolar pollen.
- Shape of the pollen: subprolate to spheroidal.
- Lobate amb of the pollen.
- Heterocolpate. Circular to lalongate os.
- Tectate exine.
- Tannins.

Fruit

- Vascular structure of the peduncle: eustele of bicollateral bundles.
- Pericycle made up of a collenchymatic sheath and an inner parenchyma.
- Calcium oxalate druses in primary cortex, external phloem, and pith of the peduncle.
- “Combretaceous” trichomes in the epidermis of the peduncle and the epicarp.
- Anomocytic stomata in the epicarp.
- Parenchymatic mesocarp.
- Sclerenchymatic endocarp (sclereids and fibers).
- Calcium oxalate druses in endocarp.

- Calcium oxalate prismatic crystals in fibers of endocarp.
- Tannins.

Seed

- Pale yellow exotesta, of cellulose walls, with drops of oil; yellowish-chestnut colored mesotesta, of sclerotic cells; yellowish-chestnut colored endotesta and exotegmen, of tracheidal cells with spiral thickenings, and pale yellow endotegmen of cells of thin, tangentially lengthened and flattened cellulosic walls.
- Cotyledons of convolute vernation, with drops of oil, bodies of aleurone, and very scanty secondary starch.
- Calcium oxalate druses in the cotyledons.
- Tannins.

The micrographical quantitative parameters of the aerial organs, with LM, are presented in Tables 1, 2, 3, and 4.

References

- Backes A, Nardino M (1999): Nomes populares e científicos de plantas do Rio Grande do Sul. São Leopoldo, Rio Grande do Sul, Unisinos.
- Barth OM, Silva SAF (1965): Catálogo sistemático dos polens das plantas arbóreas do Brasil meridional VII Thymelaeaceae, Lythraceae, Lecythidaceae, Rhizophoraceae e Combretaceae. *Mem Inst Oswaldo Cruz* 63: 255–278.
- Bassols GB, Gurni AA (1998): Posibles adulterantes del poleo (*Lippia turbinata* Griseb., Verbenaceae). *Acta Farmacéutica Bonaerense* 17: 191–196.
- Bertoni MS (1914). Las plantas usuales del Paraguay y países limítrofes 31.1. Introducción, nomenclatura y diccionario de los géneros botánicos latino guaraní. Asunción, M. Brossa.
- Cabrera AL, Dawson G (1944): La selva marginal de Punta Lara. *Rev Mus La Plata Botánica* 5: 276–382.
- Carpano SM, Carosanti V (1998): Actividad bactericida de *Terminalia australis* Cambess. “palo amarillo” (Combretaceae) frente a cepas patógenas de *Pseudomonas aeruginosa*. Suplemento de la Revista Farmacéutica, Libro de Resúmenes de las Jornadas Científicas de la Academia Nacional de Farmacia y Bioquímica, Bs. As.
- Carpano SM, Spegazzini ED, Rossi JS, Castro MT, Debenedetti SL (2003): Antifungal activity of *Terminalia australis*. *Fitoterapia* 74: 294–297.
- Castro MT, Carpano SM, Spegazzini ED, Nájera MT (2001): Leaf micrographic parameters of *Terminalia australis* Cambessedes (Combretaceae). *Bol Mus Reg Sci Nat Torino* 12: 345–354.
- Corrêa MP (1984): *Dicionário das Plantas Úteis do Brasil e das Exóticas Cultivadas*, Vol. 1. Rio de Janeiro, Ministério da Agricultura Inst. Brasileiro de Desenvolvimento Florestal.

- Dominguez JA, Molfino JF, Gallelli EL de (1928): Composición química de las plantas Argentinas. *Trab Inst Bot Farmcol Fac Cs Méd 40*: 1–75.
- Eichler AW (1867): Combretaceae. In: Martius CFP von, dir., *Flora Brasiliensis* 14, 2, Munich Oldenbourg.
- Erdtman G (1952): *Pollen Morphology and Plant Taxonomy, Angiospermae*. Stockholm, Almqvist and Wiksell.
- Erdtman, G (1969): *Handbook of Palynology*. Copenhagen, Muskgard.
- Escalante MG, Carpano SM (1977): Acción antibiótica de plantas argentinas silvestres y cultivadas. *Revista Farmacéutica* 10: 109–113.
- Exell AW (1939): The “Combretaceae” of Argentina. *Lilloa* 5: 123–130.
- Exell AW, Reitz R (1967): Combretáceas. In: Reitz PR, ed., *Flora Ilustrada Catarinense 1* (fasc. COMB). Sta. Catarina, Itajaí, pp. 1–26.
- Fabris HA (1965): Combretaceae. In: Cabrera AL, dir., *Flora de la Provincia de Buenos Aires*. Tomo IV, Parte IV. Buenos Aires Colección Científica del INTA.
- Guaglianone ER (1998): Combretaceae. In: Hunziker AT, ed., *Flora Fanerogámica Argentina*, 58: 1–8. Córdoba, PROFLOA (CONICET).
- Guaglianone ER (1999): Combretaceae. In: Zuloaga FO, Morrone O, ed., *Catálogo de las Plantas Vasculares de la Rep. Argentina II. Acanthaceae-Euphorbiaceae (Dicotyledoneae)*. Vol. 74. Monographs in Systematic Botany from the Missouri Botanical Garden, p. 621.
- Hieronymus J (1882): Plantae Diaphoricae florum argentinæ. *Bol Acad Nac Ciencias Córdoba IV*: 199–598.
- Hultin E (1965): Alkaloid-screening of plants from Boyce Thompson Southwestern Arboretum. *Acta Chem Scand* 19: 1297–1300.
- Klucking EP (1991): *Leaf Venation Patterns*, Vol. 5. Combretaceae. Stuttgart, J. Cramer.
- Lahitte HB, Hurrell JA (1995): *Las plantas de la Medicina popular de la Isla Martín García*, Vol 50. La Plata, Comisión de Investigaciones Científicas, Provincia De Aires, serie Informes, pp. 1–246.
- Lahitte HB, Hurrell JA (1997a): *Los árboles de la Isla Martín García*. La Plata, Programas Estructura y Dinámica y Ecología del no Equilibrio. Comisión de Investigaciones Científicas De la Provincia de Buenos Aires.
- Lahitte HB, Hurrell JA (1997b): *Plantas de la Costa*. Literature of Latin America (LOLA).
- Leonardis RFJ (1976): *Libro del Árbol*, Vol. II, 2nd ed. Buenos Aires, Celulosa Argentina.
- Lombardo A (1964): *Flora Arbórea y Arborescente del Uruguay*. Montevideo, Dir Pas Publ.
- Markgraf V, D’Antoni HL (1978): *Pollen Flora of Argentina*. Tucson, The University of Arizona Press.
- Metcalf CR, Chalk L (1950): *Anatomy of the Dicotyledons*, Vol I. Oxford, Clarendon Press.
- Muñoz J, Ross P, Gracco P (1993): *Flora Indígena del Uruguay. Árboles y Arbustos Ornamentales*. Montevideo, Hemisferio Sur.
- Punt W, Blackmore S, Nilsson S, Le Thomas A (1994): *Glossary of Pollen and Spore Terminology*. LPP Foundation, Laboratory of Palaeobotany and Palynology, LPP Contributions Series (1). University of Utrecht, p. 72.
- Verhoeven RL, Van der Schijff HP (1975): Anatomical aspects of Combretaceae in South Africa. *Phytomorphology* 24: 158–164.
- Vliet GJCM van (1979): Wood anatomy of the Combretaceae. *Blumea* 25: 141–223.
- Wodehouse RP (1935): *Pollen Grains*. New York, McGraw-Hill.