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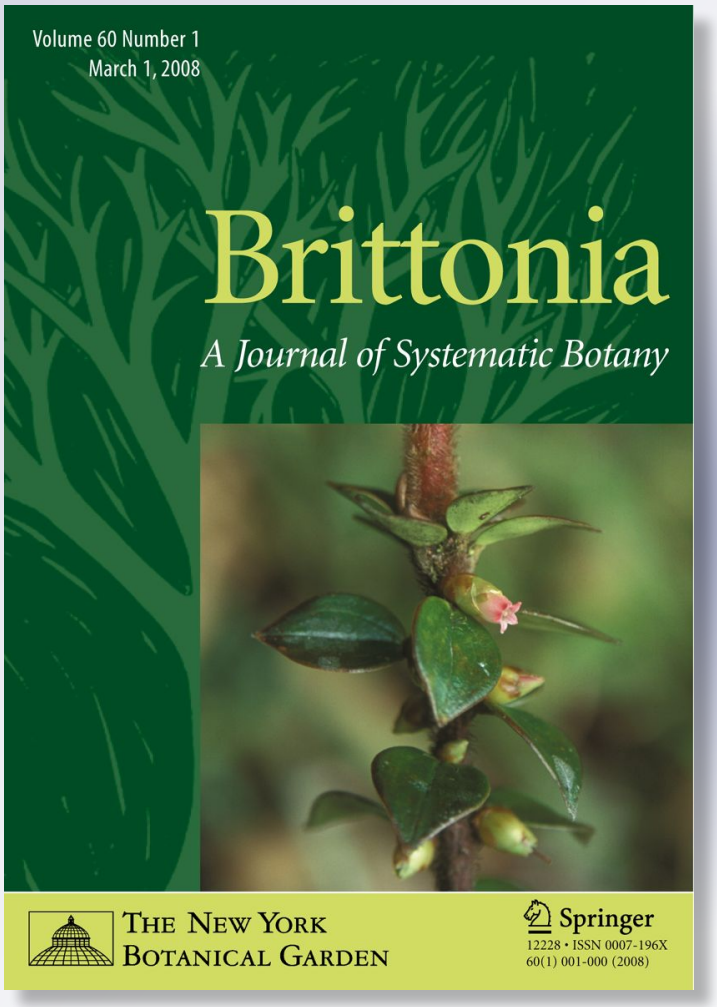

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Anatomy, pollen, and chromosomes of *Adenoa* (Turneraceae), a monotypic genus endemic to Cuba

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Abstract. The monotypic genus *Adenoa* is endemic to Cuba. Its name alludes to the presence of minute glands on the petal margin, identified in the present study as lachrymiform colleters. Here we describe the morphological, anatomical, palynological, and chromosome features that characterize *Adenoa cubensis*. The indumentum of *Adenoa* consists only of stellate trichomes. Unlike many species of the new world genera *Piriqueta* and *Turnera*, *Adenoa* lacks glandular hairs and extrafloral nectaries. *Adenoa*, *Piriqueta*, and *Turnera* share the presence of standard, sessile, and lachrymiform colleters. The leaves of *Adenoa* have xeromorphic features, which include entire, revolute blade margins, an adaxial hypodermis, and stomata restricted to the abaxial surface. The chromosome number is 2n=14, which is probably the ancestral number of the family. *Adenoa* chromosomes are similar in size to those of *Turnera*, and are larger than those of *Piriqueta*. Using the available data, we discuss relationships among the new world genera of Turneraceae.

Key Words: Emergences, floral anatomy, floral vascularization, foliar ontogeny, indumentum, leaves, pollen, seeds.

Turneraceae is a family of flowering plants consisting of 120 species in ten genera. In the new world, this family is represented by four genera: *Adenoa* (1 sp.), *Erblichia* (1 sp.), *Piriqueta* (44 spp.), and *Turnera* (142 spp.). The anatomy and chromosomes of *Piriqueta* and *Turnera* have been analyzed in several previous studies (Arbo & Fernández, 1983; Fernández, 1987; Gonzalez, 1998, 2000, 2001; Gonzalez & Arbo, 2004, 2005; Gonzalez & Ocantos, 2006; Shore et al., 2006). There is no information about chromosomes of the African genera (Shore et al., 2006).

Studies on pollen morphology of Turneraceae are scarce. The small genus *Erblichia* is the only one fully studied (Arbo, 1979). The monotypic genus *Mathurina* and a few species of *Piriqueta, Tricliceras* (*Wormskioldia),* and *Turnera* have been described by Erdtman (1966), and Arbo and Salgado (2004). Pollen dimorphism has been described in *Turnera subulata* Smith (Rama Swamy & Bahadur, 1984, 1985).

Adenoa cubensis (Britt. & Wilson) Arbo is a shrub endemic to the extreme SE of Cuba that lives in the "charrascales" of the "Sierras" at 400–750 m. It has whitish flowers about 3 cm long with exserted styles. The aim of this research was to study the anatomy, pollen, and chromosomes of *Adenoa* in order to compare the results with those from the other genera of Turneraceae. Acquiring information about the variability present in the family is a crucial step toward understanding the phylogeny.

Materials and methods

Voucher specimens: CUBA. Cuaba: Baracoa, Charrascos en los alrededores del Arroyo Maguana, Apr 2008, *Álvarez et al.* (HAJB-*55687*); Baracoa, Guantánamo, charrascos de La Cuaba, 11 Jul 2007, *González* (HAJB-

85370); Holguín, Dep. Moa, 26 May 2007, *Zuloaga et al. 9600* (SI, CTES).

Light microscopy (LM)

Leaves, buds, cotyledons, flowers in different stages of development, and seeds were fixed in FAA (70% alcohol, formalin, acetic acid, 90:5:5). Samples were dehydrated using the tertiary butyl alcohol series, embedded in paraffin (Johansen, 1940), transversely and longitudinally sectioned with a rotary microtome (Microm), and stained with safranin-astra blue (Luque et al., 1996). Leaves were cleared using the method of Dizeo de Strittmatter (1973). The leaf structure was described following the Manual of Leaf Architecture (Leaf Architecture Group, 1999), and at least ten leaves were measured. Sections were stained using IKI and Sudan IV for starch and lipid recognition (Johansen, 1940).

Pollen was prepared following Erdtman's technique (1966). Semi-permanent slides mounted in glycerin jelly (Johansen, 1940) were prepared. Polar axis (P), equatorial diameter (E), ambitus length, colpus length, porus length and width, and exine width were measured in about 30 pollen grains. Minimum, maximum, and mean of each parameter was obtained. Pollen morphology was described following Punt et al. (1994, 2007).

For cytological research, roots were pretreated in 0.002 M 8-hydroxyquinoline at room temperature for 4 h, fixed in 5:1 absolute ethanol-butyric acid and then stored in 70% ethanol. The roots were hydrolyzed in 1 N HCl at 60°C for 8 min, stained with the Feulgen technique (Feulgen & Rossenbeck, 1924), and squashed in 2% acetic orcein. Slides were made permanent using the liquid CO_2 method of Bowen (1956).

Observations, photomicrographs, and drawings were obtained with a Leica MZ6 stereoscopic microscope and a Leica DM LB2 optical microscope with a drawing tube and Canon PowerShot S80 digital photographic camera.

Scanning electron microscopy (SEM)

Organ and tissue samples for SEM observation were dehydrated in an acetone series, critical point dried (Denton Vacuum DCP-1), and sputter coated with gold-palladium (Denton vacuum sputter coater). For SEM observation of pollen grains, temporary slides were prepared on aluminum foil, dried, and sputter coated with gold-palladium. All SEM images were obtained with a Jeol LV 5800 scanning electron microscope, at the Scanning Electron Microscopy Service of the "Universidad Nacional del Nordeste," Corrientes, Argentina.

The following abbreviations are used in the text and figure captions: TS: transaction; LS: longisection; LM: optical microscopy; SEM: scanning electron microscopy; FN: floral nectary; SV: surface view.

Results

Trichomes

Morphology.—Trichomes in *A. cubensis* are stellate, with 6–18 rays (Fig. 1). Each ray consists of a single, thick-walled cell with a smooth to somewhat sculptured surface. The basal part of the rays are interconnected by simple pits along the stalk of the trichome. These hairs are distributed throughout the plant body (Fig. 1D, E). They are scarce on the adaxial surface of the leaves (Fig. 1B) and plentiful on the abaxial surface (Fig. 1A).

Ontogeny.—Each hair originates from a protodermal cell that elongates and divides anticlinally into as many cells as rays the trichome will have (Fig. 1F–K). These cells grow radially, soon separating from one another so that the rays of the trichome are discrete. Epidermal cells adjacent to the trichome base sometimes expand concomitantly with trichome development and form a pedestal that slightly elevates the hair (Fig. 1C, D).

Colleters

Anatomy.—Colleters of *A. cubensis* are "emergences" and cannot be regarded as epidermal structures, because they develop from both protoderm and ground elements. They are composed of a subepidermal, pluricellular, parenchymatous axis sheathed with a palisade epidermis (Fig. 2G–I). They lack vascularization. The cuticle is smooth, thick at the base, and thin at the apex.

Three types of colleters are recognized (Gonzalez, 1998): 1) Standard—cylindrical to claviform, with a rounded apex (Fig. 2A, G), present on young stems, petioles,

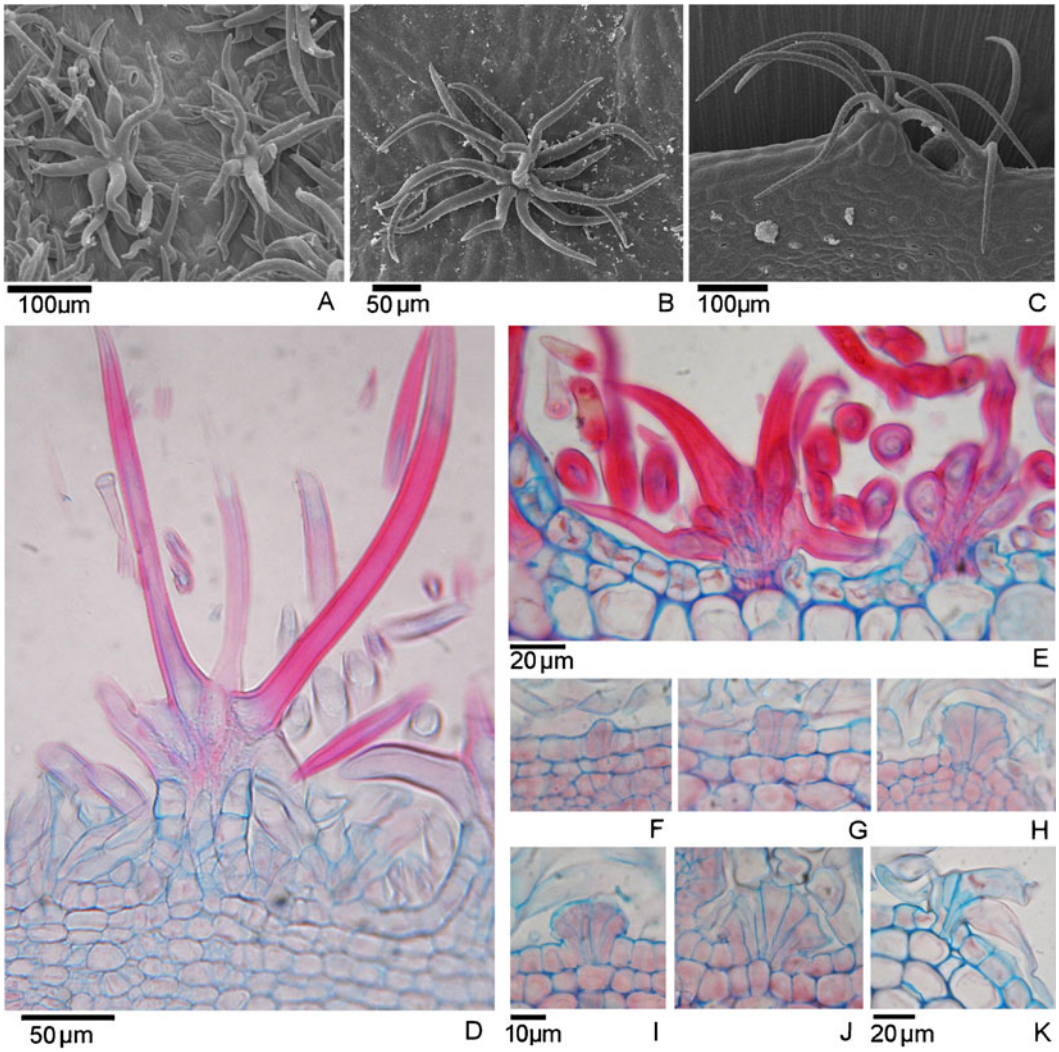

Fig. 1. SEM and LM photographs of the indumentum, stellate trichomes. **A**. Abaxial leaf surface. **B**. Adaxial leaf surface. **C**. Cotyledon margin. **D**. Ovary surface. **E**. Adaxial leaf surface. **F–K**. Ontogeny of trichome on ovary surface.

peduncles, prophylls and anther apices; 2) Lachrymiform—tear-shaped, with an acute apex (Fig. 2I), abundant along the margin of petals (Fig. 2B–D), scarce adjacent to the principal veins on both surfaces of leaves (Fig. 2K); and 3) Sessile—subspherical, with a wide base, short axis and round apex, present on the adaxial epidermis of leaves (Fig. 2F, H).

The colleters of stems and the leaf abaxial surface are hidden within the indumentum (Fig. 2F, K), whereas those of the anthers (Fig. 2A) and petals (Fig. 2D) are clearly exposed. Colleters are also present along the margin of cotyledons, where the abundant slightly viscous secretory product is visible (Fig. 2J).

Ontogeny.—The colleter primordia consist of protoderm and subtending ground meristem. A group of protoderm cells dividing anticlinally lead to the formation of palisade epidermis. The ground meristem undergoes anticlinal and periclinal divisions to form the colleter axis (Fig. 2L–O).

The differences in the development of diverse types of colleters are determined by

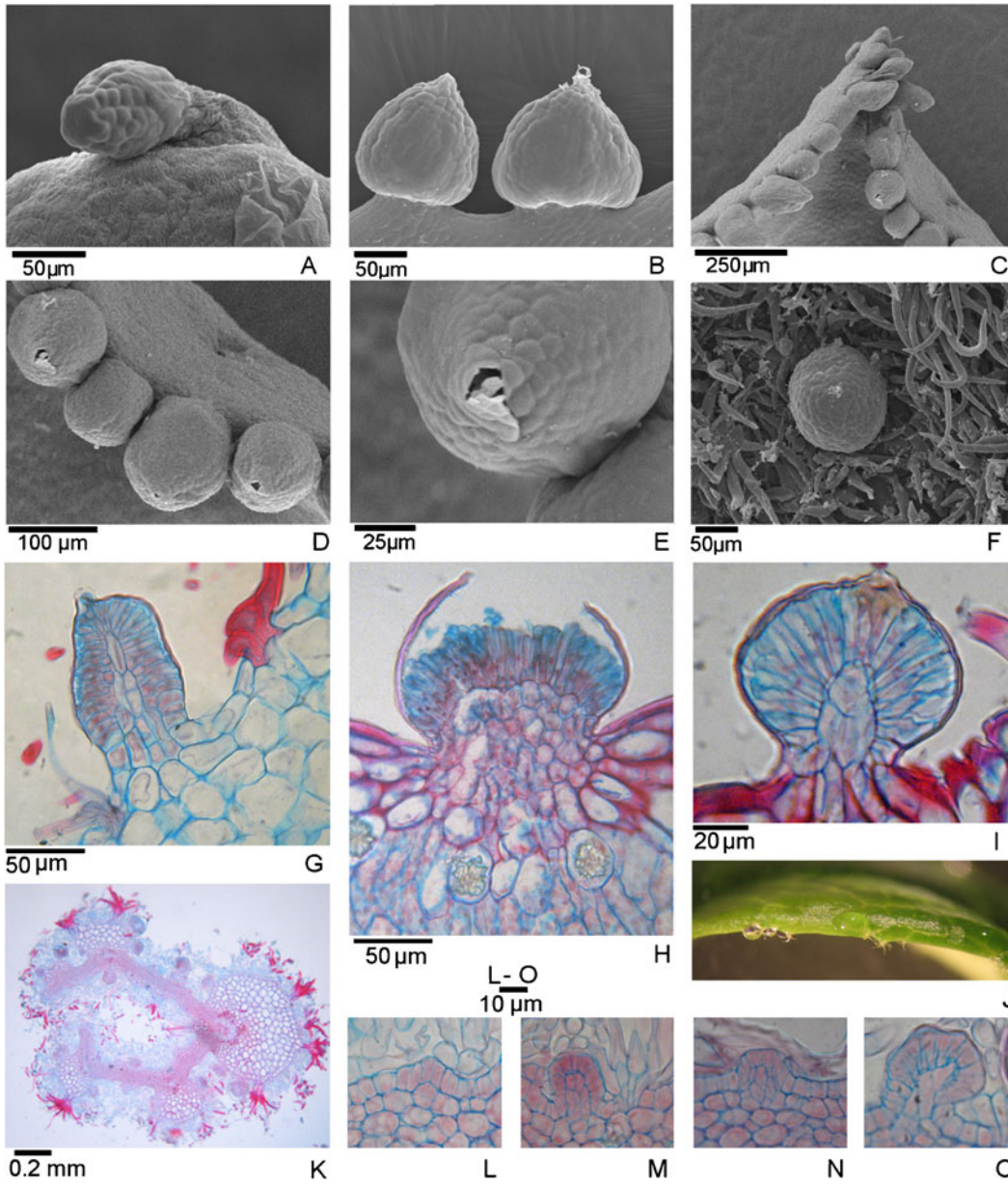

FIG. 2. SEM and LM photographs of colleters. **A**. Standard colleter at anther apex. **B**. Lachrymiform colleters on petal margin. **C**, **D**. Petal margin with colleters. **E**. Detail of colleter apex showing broken cuticle. **F**. Sessile colleter on abaxial leaf surface. **G**. Standard colleter. **H**. Sessile colleter. **I**. Lachrymiform colleter. **J**. Cotyledon margin with colleters showing drops of secretion. **K**. TS of leaf primordia showing indumentum and colleters. **L–O**. Ontogeny of colleter on abaxial leaf surface.

the differential expansion of the colleter axis. Developmental maturation of colleters precedes mesophyll differentiation. In active colleters, the secretion flows at the apex through interruptions in the cuticle (Figs. 2B, E, G).

LEAVES

Morphology.—Leaves are simple, coriaceous, and alternate in *A. cubensis*. The petiole is cylindrical. The blades are sym-

metrically elliptic or oblong-obovate in outline, the mean LW ratio is 3.75:1. They have a cuneate or attenuate base and an acute or obtuse apex; the margin is entire and slightly revolute. The main vein is straight. Secondary venation is brochidodromous and tertiary venation is randomly reticulate. The fourth-order veins have regular, polygonal reticulate configuration such that they delimit well-developed, 4 or 5-sided areoles. The ultimate veins have free, unbranched endings. The marginal venation includes a fimbrial vein (Fig. 3A, B).

Anatomy.—The adaxial epidermal cells in surface view are polygonal in outline (Fig. 3D). In transverse section, the adaxial epidermis is uniseriate and composed of square cells that have thick, lignified tangential walls and narrow radial walls. The adaxial surface has a thick cuticle and lacks stomata (Fig. 3F–H); a sparse indument of stellate hairs is mainly restricted to areas adjacent to the veins. The abaxial surface is covered with dense stellate hairs, which obscure the epidermal cells (Fig. 1A). Leaves are hypostomatic, with a stomatal index of (12.8–) 14.7 (−17.5). Stomata are anomocytic and surrounded by 4–6 epidermal cells (Fig. 3E); they are restricted to the areoles (Fig. 3J).

The adaxial hypodermis is mostly uniseriate, but extends to 5 or 6 layers in depth adjacent to the vascular bundles (Fig. 3F, G). The hypodermal cells are colorless and have thick walls with large primary pit fields. Idioblasts, each including a large druse crystal, are scattered between the hypodermis and mesophyll (Fig. 3H). The mesophyll is dorsiventral (Fig. 3F); the palisade parenchyma is irregularly arranged and 1–4 cells deep. The network of major and minor veins protrudes prominently on the abaxial surface of the lamina, and delimits depressed areoles (Fig. 3C).

Venation.—The midvein is prominent, and includes a large C-shaped collateral bundle and a small, superimposed, inverted bundle (Fig. 3G). Veins of 1st to 4th order are abaxially positioned. The smaller vascular bundles are collateral, with a parenchymatous bundle sheath (Fig. 3F).

Foliar ontogeny.—The lamina of leaf primordia is 6 or 7 cell layers in depth (Fig. 3K, L). The cell divisions start at the middle layers where the vascular bundles originate. Following the vascularization pattern, the cells of the upper hypodermis divide repeatedly to build the hypodermal extensions towards the vascular bundles. The innermost cells retain a thin wall and each develops a large druse crystal within. The abaxial hypodermal cells develop thick walls only below the veins.

Flowers

Morphology.—Flowers are axillary and solitary, with a free peduncle (1.5 cm), a short pedicel (0.5 cm), and a pair of sessile, opposite prophylls (bracteoles) at the joint. Flowers are pentamerous and actinomorphic, with the proximal portion of the sepals and petals united into a perianth tube. The stamens are attached at the base of the perianth tube. Each bears a dorsifixed anther that dehisces via longitudinal slits. The aestivation of the calyx lobes is quincuncial, while that of the petals is imbricate. The gynoecium is tricarpellate and the ovary is syncarpous (paracarpous), superior, one-celled, with parietal placentas and anatropous ovules. Three free styles, each terminating in a shallowly lobed stigma, extend from the ovary. Pistils with four carpels are sometimes found.

The predominant reproductive system in Turneraceae is distyly. However, there are homostylous genera and species. *Adenoa* has homostylous flowers (Arbo, 1977; Shore et al., 2006).

Peduncle, pedicel, and receptacle.— The epidermis is uniseriate, with stomata and abundant stellate hairs. The cortical parenchyma lacks intercellular spaces. The cells of the outer layers have chloroplasts, and some of them have druses. The basal third of the peduncle has colleters with smooth cuticle and the cells of the axis slightly lignified. The peduncle elongates during fruit development, reaching 2.3–2.5 cm when the fruit is ripe. The floral pedicel is very short, 2–5 mm long. Colleters are absent in the pedicel and receptacle.

Prophylls.—These have marginal colleters at the base. In transverse section they have a uniseriate epidermis with abundant hairs and mesophyll composed of homogeneous chlorenchyma. Druses are commonly

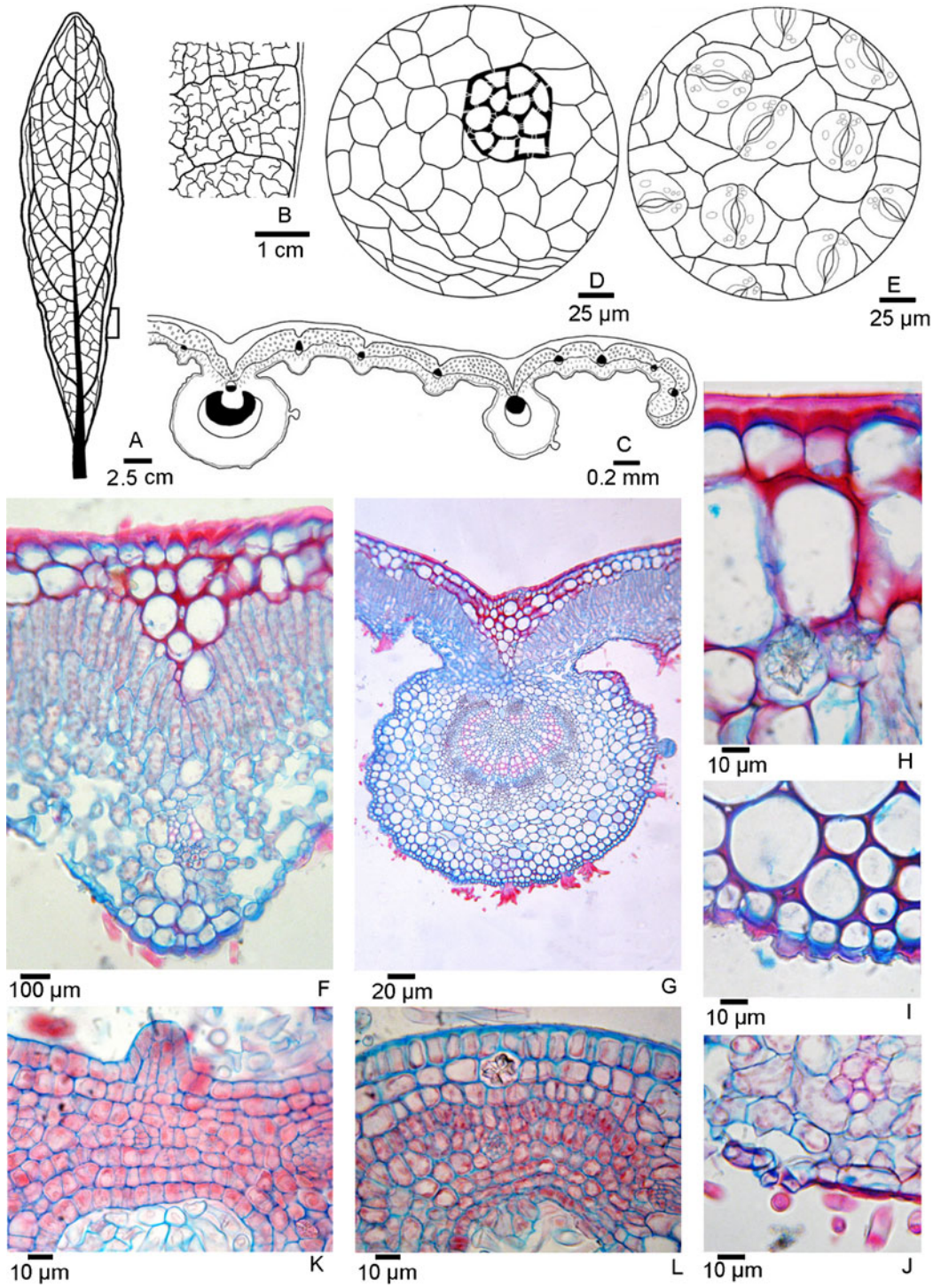

Fig. 3. Leaf lamina. **A**. Foliar venation. **B**. Detail of venation. **C**. Lamina, TS (symbols according to Metcalfe & Chalk, 1957). **D**. Adaxial epidermis, SV. **E**. Abaxial epidermis, SV. **F**. Lamina, TS. **G**. Median vein, TS. **H**. Adaxial epidermis and hypodermis. **I**. Abaxial epidermis and hypodermis of median vein. **J**. Abaxial epidermis and hypodermis of areoles. **K**. Foliar primordium, TS. **L**. Young leaf, TS.

included. The vascular supply is embedded (Figs. 4A, 7D).

Calyx lobes.—Each calyx lobe has five prominent veins, as well as some secondary ones; the epidermis is uniseriate, the cells of the adaxial surface have narrow walls, and those of the abaxial surface have thick walls and striate cuticle; both surfaces show plentiful trichomes. The mesophyll is made up of chlorenchyma cells with slightly thickened walls (Fig. 4B).

Corolla—The petals are mostly glabrous. In transection, the petals have a uniseriate epidermis, with stomata on the abaxial surface; the cuticle is thick and smooth (Fig. 4C). The mesophyll is composed of rounded parenchyma cells with small intercellular spaces.

Perianth tube/nectary.—The outer surface is densely hairy and the inner surface consists of nectariferous tissue throughout (Fig. 4D). The inner epidermis and subepidermal parenchyma appear to be consistent with a glandular function, making up a nectary. The epidermis is uniseriate, composed of tanniferous cells with thin and smooth cuticle. The stomata are anomocytic (Fig. 4F), with a small or absent substomatal chamber (Fig. 4E). The parenchyma has polyhedral cells lacking intercellular spaces; these cells have prominent nuclei, thin walls, and dense granular cytoplasm with abundant simple or compound starch grains (Fig. 4D, E).

Androecium.—The filaments are subterete to triangular in transection, each with a uniseriate, glabrous epidermis surrounding a

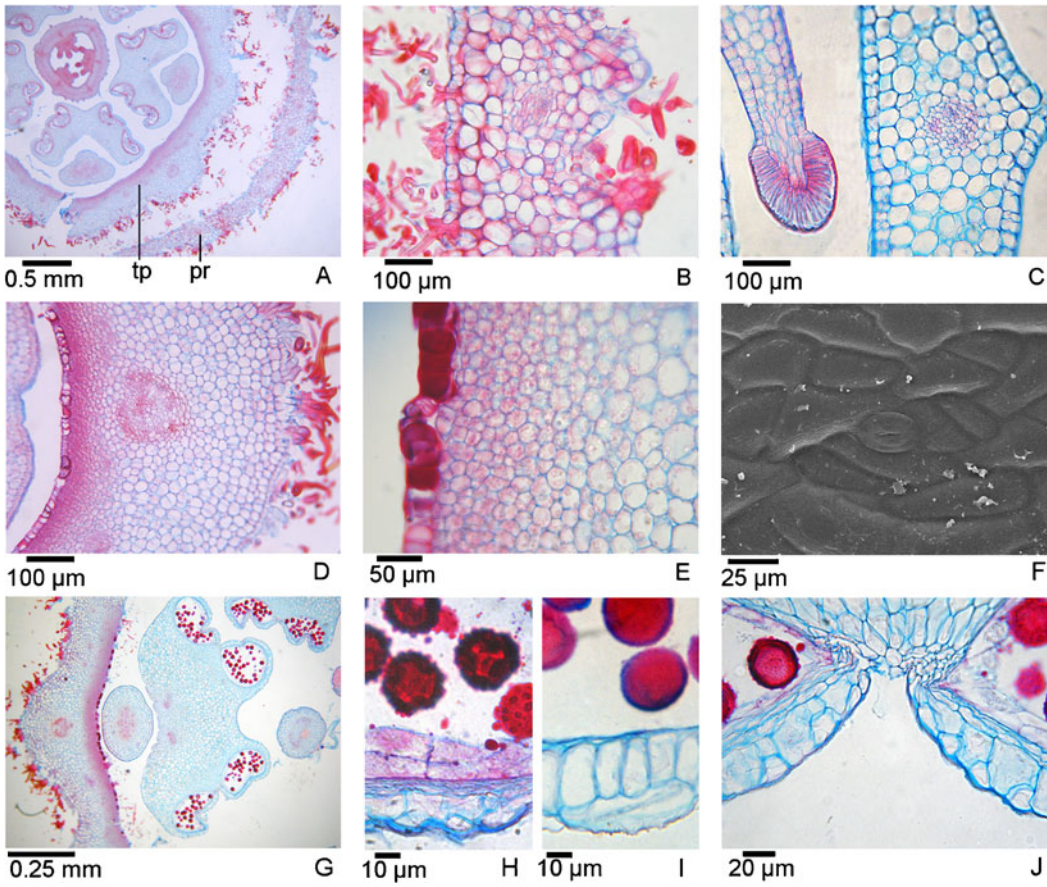

Fig. 4. LM photographs of reproductive structures: perianth and androecium. **A**. Flower, TS. **B**. Sepal, TS. **C**. Petal, TS (middle area and margin with colleter). **D**. Perianth tube, TS. **E**. Detail of floral nectary. **F**. SEM of stomata on floral nectary surface. **G**. Mature anther, TS. **H**. Wall of young anther and microspores covered by pollenkitt. **I**. Wall of mature anther and pollen grains. **J**. Stomium, TS. prophyll (pr); perianth tube (tp).

compact cylinder of parenchyma that includes a central, concentric vascular bundle. The anthers are introrse with two bilocular thecae (Figs. 4A, G; 7J–L). The young anthers have an epidermis, an endothecium, 2–4 parietal layers and a bilayered, secretory tapetum that releases numerous Ubish bodies among the pollen grains (Fig. 4H). At or near anther maturity, the parietal layers become crushed, the innermost layer of the tapetum disintegrates and the outermost tapetal layer collapses. Prior to anthesis, the mature anther has four microsporangia that are surrounded by an endothecial layer and a discontinuous epidermis. The epidermal cells have a convex external wall covered by a thin striate cuticle (Fig. 4I). The endothecial cells have weakly lignified annular wall thickenings. The connective tissue is parenchymatous, and the stomium is made up of very small cells (Fig. 4J).

Gynoecium.—The outer epidermis of the ovary is densely covered with stellate hairs (Figs. 4A; 5A). The glabrous inner epidermis has few stomata. The styles are glabrous and solid, consisting primarily of parenchymatous tissue. Each includes up to 12 small vascular bundles. The transmitting tissue is continuous from the placentas to the stigmas (Fig. 5B, C).

Ovules.—The ovules are anatropous, bitegmic, and crassinucellate (Figs. 5D,E, 6A). The outer integument is two-layered, and the inner integument is three-layered (sometimes four-layered; Fig. 6B). The nucellus is somewhat curved along the raphe, the vascular bundle is unbranched, and the chalaza is bulky. At the region where the hilum will differentiate, there is a collar-shaped bulge that will develop into an aril (Figs. 5D; 6A).

Ovule ontogeny.—At initiation, the ovule primordia appear as hemispherical bulges on the placentas. Ovules are anatropous, so the apex curves soon towards the placenta. The integuments differentiate by anticlinal and oblique divisions of the dermal layer (Fig. 6D). The inner integument is annular, surrounding the primordia, while the external one is not developed between the raphe and the nucellus. The external integument reaches its full length after the ovule turns 180° (Fig. 6E, F).

Early in ovule development, the integuments are each composed of two layers, but subsequently the cells of the inner layer of the inner integument undergo periclinal divisions, giving rise to a third layer. The aril has a dermal origin (Fig. 6F). When the differentiation of the procambium takes place, long and slender cells with dense cytoplasm arise along the raphe. The bulky chalaza is composed of small cells which dense cytoplasm (Figs. 5E, 6F). The micropyle is delimited by the inner integument and the external (antirapheal) segment of the outer integument. Mature ovules therefore have well developed endostome and lack a clearly defined exostome (Fig. 6A).

Floral vascularization.—The vascular system at the base of peduncle is an ectophloic siphonostele. Two collateral traces, which supply the main veins of the prophylls, are the most proximal traces to depart from the stele (Fig. 7A, B). Another four lateral veins run all along the peduncle (Fig. 7C) and split repeatedly (Fig. 7D).

Ten traces branch from the stele at the base of the receptacle. Five of these supply the sepals; they are collateral but become concentric distally. The other five supply the petals (Fig. 7E). Next, five concentric bundles, which innervate the stamens, start off (Fig. 7F). The following whorl is composed of six traces: three collateral bundles are the dorsal bundles of the carpels and three groups of two or three small traces are the marginal bundles (Fig. 7G). The remnant of the stele consists of numerous small bundles that gather in three groups; these are the placentary bundles that will innervate the ovules (Fig. 7H).

Within the perianth tube, branches of the sepal and petal traces are much ramified near the inner surface of the tube, forming a discrete vascular net beneath the nectariferous tissue (Fig. 7I, J) and the minor veins of the calyx lobes. At the throat of the perianth tube, each petal trace branches radially and tangentially to form a group of five bundles. The two most peripheral will innervate the margins of the two adjacent calyx lobes (Fig. 7K), whereas the other three become the median and lateral petal veins (Fig. 7K).

Each stamen bundle is unbranched from the base of the filament to the connective, where the bundle splits to supply the micro-

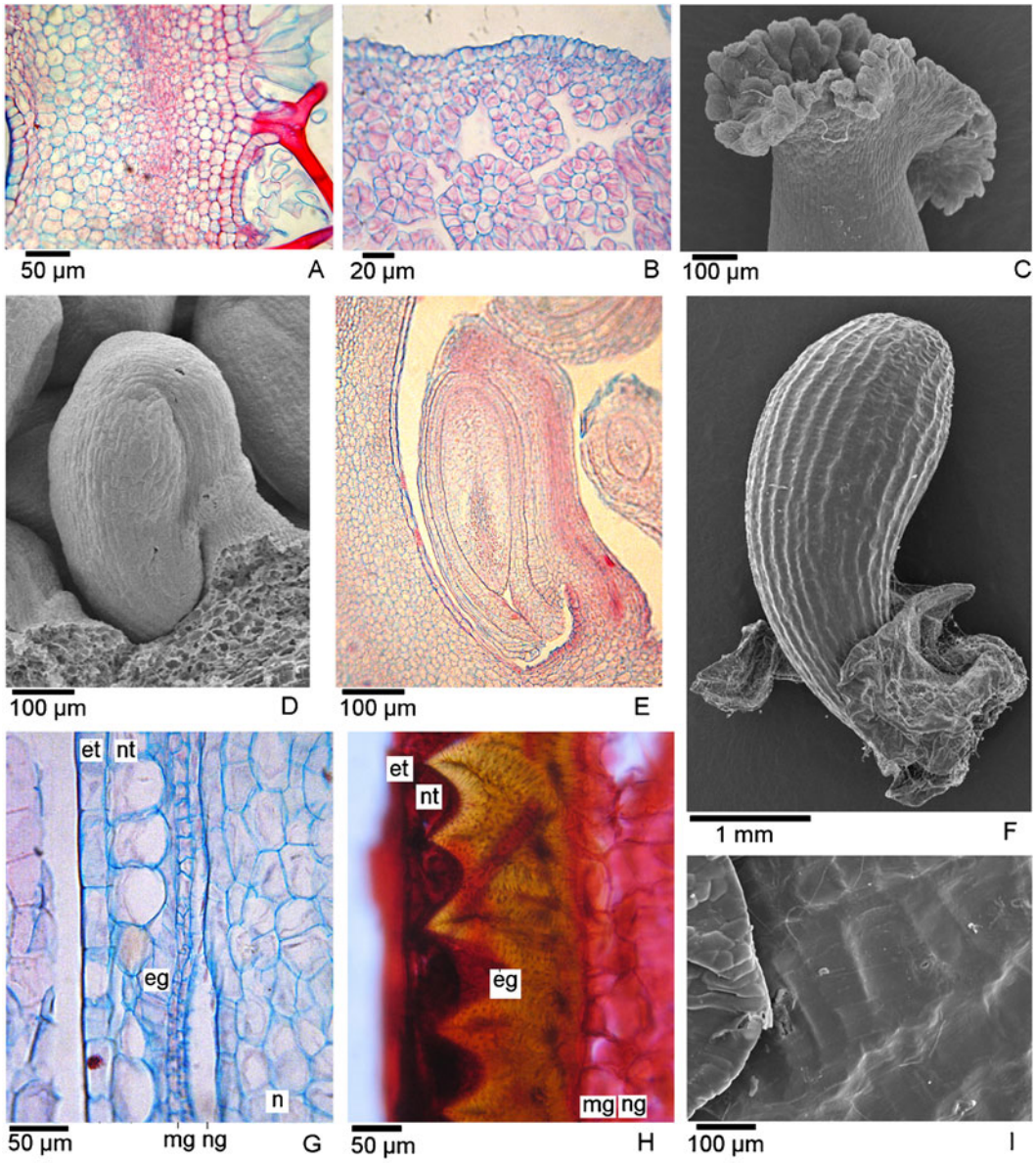

Fig. 5. LM and SEM photographs of reproductive structures: gynoecium and seed. **A**. Carpellary wall, TS. **B**. Stigma, TS. **C**. Stigma. **D**. Ovule. **E**. Ovule, LS. **F**. Seed. **G**. Detail of young seed, LS. **H**. Detail of mature seed, LS. **I**. Details of episperm and aril. Abbreviations: exotegmen (eg); exotesta (et); mesotegmen (mg); nucellus (n); endotegmen (ng); endotesta (nt).

sporangia (Fig. 7I–K). The dorsal bundles of the ovary run along the carpel wall; at the base of the styles they split in up to ten small bundles, which are arranged in a circle (Fig. 7J–L). At the base of the stigmas the bundles gradually disappear. The marginal bundles of the ovary split several times starting off as small bundles that innervate the carpel wall; they end at the apex of the ovary.

Seeds

The seeds are small, obovoid, and curved, with a rounded chalaza. The episperm is dark-

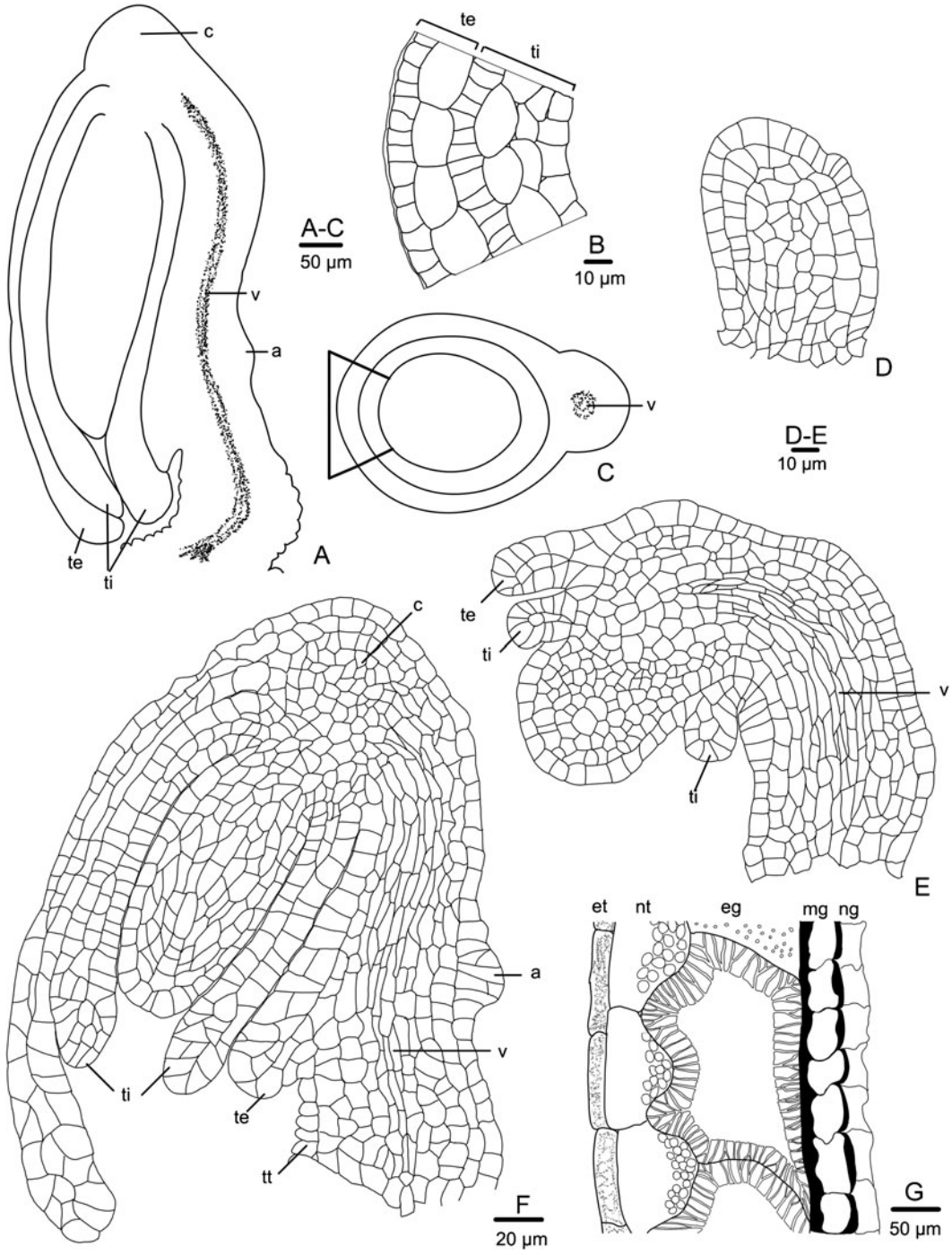

Fig. 6. Diagrams of ovule and seed. **A**. Ovule. **B**. Integuments, TS of area indicated in **C**, **D**. Ovular primordium. **E**. Initiation of integuments at ovular primordium. **F**. Young ovule with aril initiation. **G.** Episperm, LS in mature seed. Abbreviations: aril (a); chalaza (c); exotegmen (eg); exotesta (et); mesotegmen (mg); endotegmen (ng); endotesta (nt); external tegument (te); internal tegument (ti); transmitting tissue (tt); vascular bundle (v).

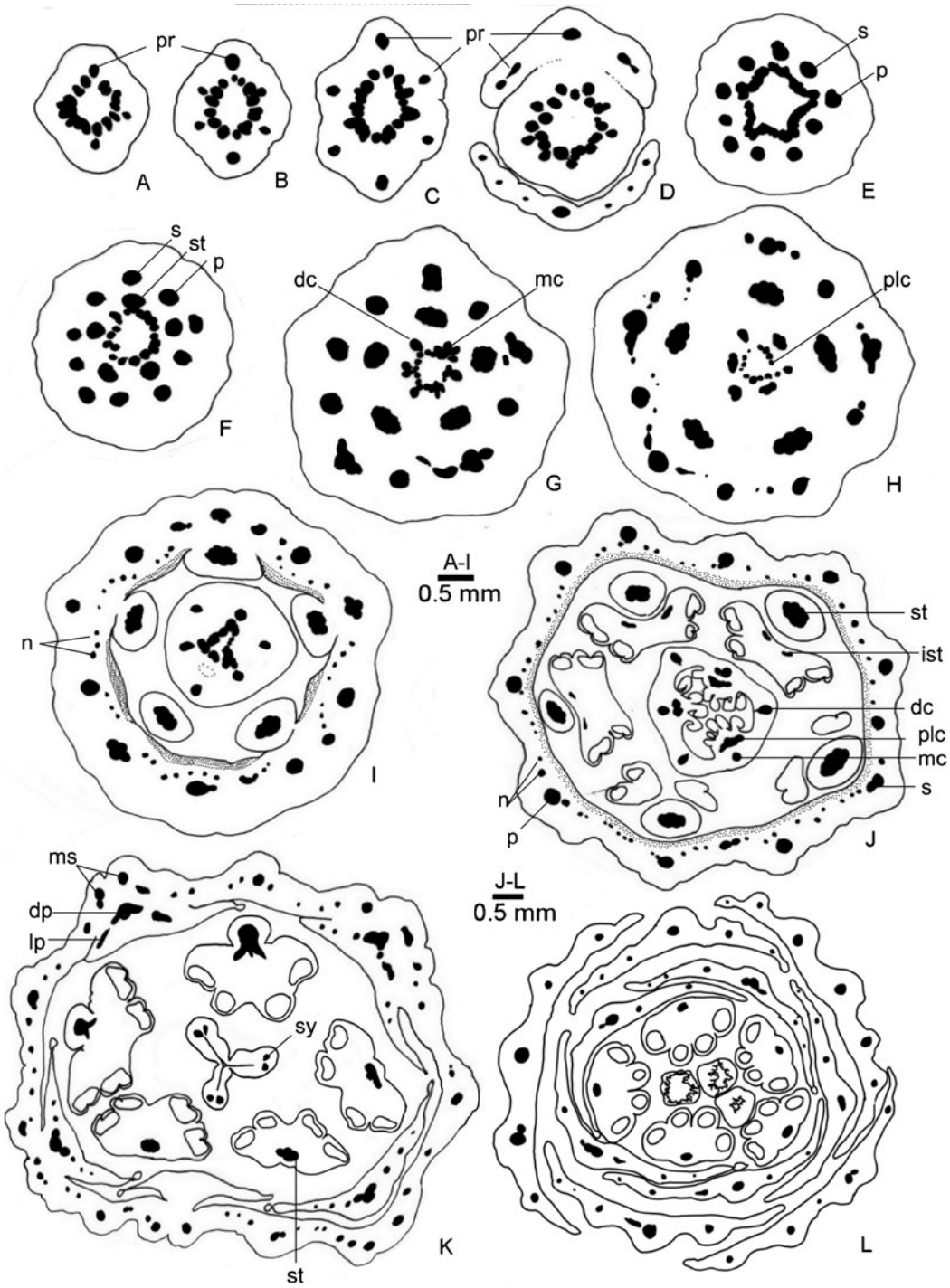

Fig. 7. Floral vascularization, TS at different levels. **A**. Peduncle. **B–D**. Pedicel. **E–H**. Receptacle. **I**, **J**. Ovary and perianth tube. **K**. Perianth tube, anthers and styles. **L**. Sepals, petals, stamens and stigmata. Abbreviations: dorsal carpel bundle (dc), dorsal petal bundle (dp); inverted stamen bundle (ist); lateral petal bundle (lp); marginal carpel bundle (mc); marginal sepal bundle (ms); nectary bundles (n); petal bundle (p); placenta bundle (plc); prophyll bundle (pr); sepal bundle (s); stamen bundle (st); stigma (sy).

brown to blackish, glabrous and reticulate-striate, with rectangular areoles arranged in longitudinal rows; the transverse ridges are barely perceptible (Fig. 5F, I).

Testa.—The testa is made up of both layers of the ovule external integument (Figs. 5G, H, M; 6G). In surface view, the cells of the exotesta are polyhedral to rounded, usually elongate along the raphe, with cellulosic walls, and the thickened external wall covered by a smooth and thin cuticle; the stomata are scattered and anomocytic (Fig. 5I). The endotesta layer undergoes deep changes during the seed differentiation; it sets the size and shape of the areoles. Each cell matches with a reticule areole of the episperm; in transection the cells are hemispherical, shield-shaped, with abundant starch grains (Fig. 6G). These cells are undersized at the exostome and chalaza, so the reticule areoles are consequently smaller in those parts.

Tegmen.—The tegmen is made up of the three layers of the internal integument of the ovule (Figs. 5G, H; 6G). The exotegmen, consisting of sclereids, is the mechanical layer of the seed. The sclereids make up the muri of the episperm reticule. In longitudinal section they are elongated, radially arranged, and deltoid, triangular or rhomboid in surface outline. Sclereids are of different lengths, matching the shape of the endotestal cells; the cell wall is very thick and lignified, with simple and branched pits; lumina are reduced and sometimes obliterated (Fig. 6G). At the endostome, the sclereids are relatively longer, straight, and parallel; they collectively form a beak, stretched out towards the funicle. At the chalaza the sclereids are not developed. The mesotegmen and endotegmen are composed of small cells, quadrangular or rectangular in transection; the cells of the mesotegmen have thick tangential walls (Fig. 6G).

The aril is inserted at the hilum. It consists of several layers of cells that contain abundant fatty compounds. The surface of the external layer is smooth (Fig. 5F, I).

Pollen

Light microscopy.—The pollen is large in size, *P*=53 (55.7) 59 μm, *E*=44 (46.5) 50 μm, P/E=114 (119) 131, with a subcircular amb (Fig. 8C, D), subprolate in shape (Fig. 8A, B); isopolar and radiosymmetric; 3-colporate, colpi are long and linear, up to 55 μm long; ora are lolongate (Fig. 8B), with margins poorly outlined; the apocolpia measure about 40 μm.

The exine is 3–6 μm wide; the nexine is 1 μm thick all over the grain. In contrast, the sexine is 3 μm wide at the poles and thickens towards the apertures, being 5–6 μm thick at the pore. The sexine is semitectate, microreticulate and supra-verrugate, muri are simplicolumellate (Fig. 8A). Warts of different size and shape, relatively isodiametric, are scattered all over the grain (Fig. 8A–D).

SEM.—The microreticule is homobrochate; lumina are regular, 0.4–0.6 μm in diameter; muri are about 0.5 μm wide (Fig. 8H, I). Warts are supratectal, of variable size (Fig. 8H), 2–4 μm diam. and irregular shape (Fig. 8E–G). The mature pollen grains are covered by pollenkitt (Fig. 8J).

Chromosomes

The cytological research was carried out using root-tips obtained from two month old germinating seeds. The cells analyzed show 14 chromosomes, so the basic chromosome number of *Adenoa* is *x*=7 (Fig. 9). The chromosome size is between 1.11–2.66 μm.

Discussion

The information gathered about anatomy, pollen and chromosomes makes it possible to compare the American genera of Turneraceae. There is no information on the subject about the African genera of the family.

Indumentum.—The taxonomic value of the indumentum is significant at intergeneric and infrageneric levels. *Adenoa* has stellate hairs (all rays of similar length). *Piriqueta* has porrect-stellate hairs (with a robust longer central ray) together with stellate and simple hairs. *Erblichia* and *Turnera* have simple hairs, although a few species of *Turnera*, belonging to different series of the genus, do have stellate hairs (e.g., *T. blanchetiana*, *T. cearensis*, *T. hermannioides*, *T. lamiifolia*, *T. oculata*, *T. sidoides* subsp. *sidoides*, *T. revoluta*; Arbo, 1979, 1995, 1997, 2000, 2005, 2008; Gonzalez & Arbo, 2004). Glandular hairs are most common in *Piriqueta* and *Turnera*, but have not been observed in

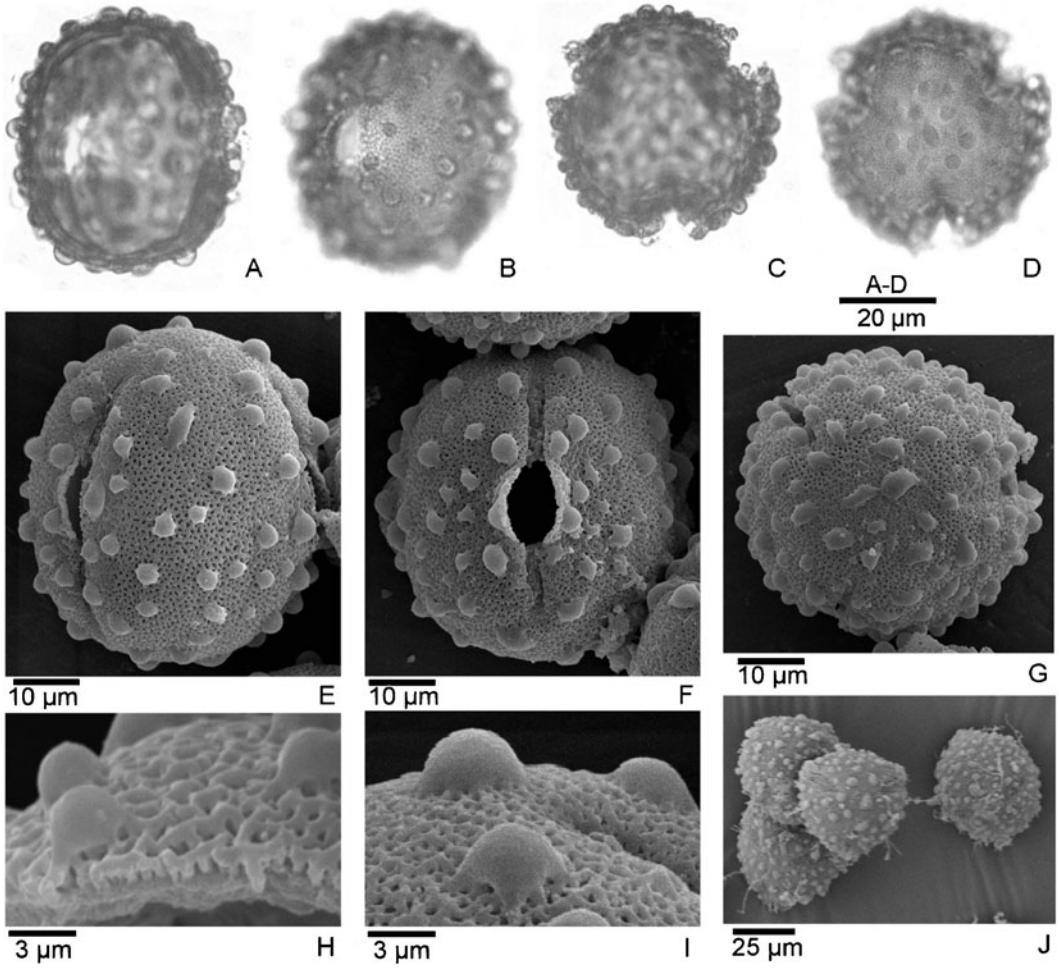

Fig. 8. LM and SEM photographs of pollen grains. **A**. Equatorial view, optical section. **B**. Equatorial view, upper focus showing the lolongate endoaperture. **C**. Polar view, optical section. **D**. Polar view, high focus. **E**. General view. **F**. Detail of aperture. **G**. Polar view. **H**. Exine section, murus simplicolumellate. **I**. Detail of exine showing the microreticule and the supratectal verruca. **J**. Pollen grain covered with pollenkitt.

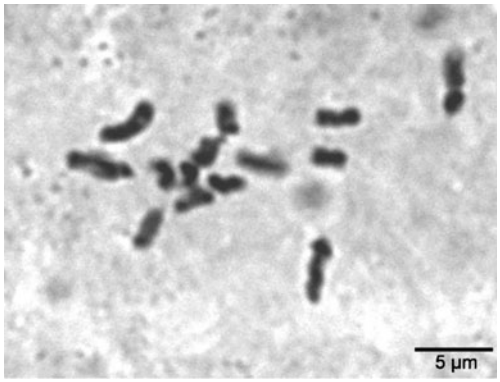

Fig. 9. Somatic chromosomes, 2n=14.

Erblichia (Arbo, 1979) or *Adenoa* (Arbo, 1977). The present study confirms they are lacking in *Adenoa*.

Colleters.—In *Turnera* and *Piriqueta* colleters are usually found on developing organs. *Adenoa* was so named because colleters are distinctively positioned along the petal margin (from the Greek *aden*-, gland, *oa*, margin; Arbo, 1977). This is a unique character state, present only in this genus of Turneraceae. In this study, we found that colleters occur not only on the petals, but are also distributed on all major organs of the primary body of the plant. Standard colleters are found on the vegetative organs of

Adenoa; these are widely distributed in *Turnera*, being the only type present in the series *Papilliferae*, *Capitatae, Turnera*, and *Salicifoliae* (Gonzalez, 1998). In *Piriqueta* they are found in a few species (*P. racemosa*, *P. cistoides*, *P. suborbicularis* and *P. taubatensis*) that do not have setiform glandular hairs (Gonzalez, 1998; Gonzalez & Arbo, 2004). Lachrymiform colleters are the type present along the margin of the petals of *Adenoa*; in *Piriqueta* they are restricted to the species with setiform glandular hairs (Gonzalez, 1998). In *Adenoa*, sessile colleters are restricted to both surfaces of the leaves. They are widespread and variably located in species of several series of *Turnera*: at the apex of stipules or replacing them, on the basal or apical teeth of the leaves, or at the inner face of prophylls. In *Piriqueta* they are found on the apical rounded teeth of the leaves of two species (*P. suborbicularis* and *P. taubatensis*; Gonzalez, 1998).

Earlier analysis of morphological variation led to the conclusion that lachrymiform colleters are the least specialized type (Gonzalez, 1998). These are known in *Piriqueta*, now they have been found in *Adenoa*.

Leaves.—The lamina anatomy of *Adenoa* is distinctive relative to most other Turneraceae in having a prominent adaxial hypodermis that extends towards the main vascular bundles (but never forming bundle sheath extensions). The major network of veins protrudes abaxially, and the stomata are restricted to the minor venation areoles. In *Turnera* and *Piriqueta*, most species have mesophytic leaves with dorsiventral mesophyll and no hypodermis. However, two species of *Turnera*, *T. genistoides* and *T. revoluta*, have ericoid leaves (narrow, revolute, hypostomatic). In the latter species, the adaxial epidermis is glabrous, composed of sclereids, and underlined by a hypodermis of tanniferous cells. These characters, together with stomata that are hidden by the dense indumentum (Gonzalez, 2000), resemble the structure of *Adenoa*. This configuration is typical of plants growing in sunny environments, often xerophytes (Fahn & Cutler, 1992), suggesting that the shared similarities might be adaptive rather than due to shared history.

The adaxial epidermal cells of *Adenoa* have straight or slightly curved anticlinal walls in surface view (Stace's types 1 and 2; Stace, 1965), like those of most species of *Turnera* and *Piriqueta* (Gonzalez, 2000). The mean stomatal density of *Adenoa* is lower than that reported for *Turnera*, (9–) 17.8 (−23), and *Piriqueta*, (16–) 20.8 (−26) (Gonzalez, 2000). *Adenoa* also lacks the EFN characteristics of many species of *Turnera* and some of *Piriqueta* (Gonzalez & Arbo, 2005; Gonzalez & Ocantos, 2006).

Flower.—A morphological sequence was set through the analysis of the floral traits of *Piriqueta* and *Turnera* (Arbo, 2009), now we include *Adenoa* and *Erblichia* in that analysis. The floral peduncle and the pedicel are developed and free in *Adenoa*, *Erblichia*, *Piriqueta*, and a few series of *Turnera*. In other series of *Turnera* the peduncle is frequently adnate to the petiole, resulting in epiphyllous flowers; the pedicel is absent in most series of *Turnera*. The perianth tube is absent in *Erblichia*, while in *Adenoa*, *Piriqueta*, and several series of *Turnera* it is well developed. In two series of *Turnera* there is a floral tube, composed of the basal parts of the calyx, corolla, and stamens. Taking into account the combination of characters of each genus, *Adenoa* would be close to the base of this morphological sequence (Arbo, 2009), more simple than *Piriqueta* but more well developed than *Erblichia*, which lacks a perianth tube.

The aestivation of the calyx is quincuncial in all the American genera, while that of the corolla is imbricate in *Adenoa* and contorted in the other genera. In *Erblichia*, *Piriqueta*, and *Turnera* the adaxial face of the calyx lobes is glabrous (except in *P. scabra* Urb. with some sparse simple hairs) while in *A. cubensis* the adaxial surface is tomentose.

The vascular system of the flowers of *Adenoa* matches the basic plan observed in *Piriqueta* and *Turnera* (Gonzalez, 1993, 2000, 2001), although it is simplified because it shows no complex traces (sepal-stamen) like those found in *Piriqueta* and *Turnera*. In *Piriqueta* and *Turnera*, the staminal bundles run along the filament and end at the connective, whereas in *Adenoa* two inverted bundles originate and run along the basal segment of the anther.

Floral nectary.—The anatomical characters of the nectariferous tissue match those described by other authors (Fahn, 1952, 1953, 1979, 1988; Bentley & Elias, 1983; Nepi, 2007); according to the histological classification of Vogel (1977), the floral nectary is of the mesenchymatic type. In *Adenoa*, like in *Piriqueta* and *Turnera*, secretion takes place via non-functional stomata or nectar slits (Gonzalez, 2001; Gonzalez & Arbo, 2005).

In *Adenoa* the nectariferous tissue coats the inner surface of the perianth tube. In some species of *Piriqueta*, the nectaries are located at the base of the perianth tube, while in other species of *Piriqueta* and some *Turnera*, the nectariferous tissue is located on the back of the staminal filaments (Gonzalez, 1993, 2000, 2001). In many species of *Turnera* the nectaries are restricted to the filaments, and the most complex case is the presence of nectariferous pockets in the series *Anomalae* and *Turnera* (staminal filaments are adnate along their margins to the petal claws up to the throat, forming nectariferous pockets between each filament and the corresponding sepal; Arbo, 2009). This sequence matches the acrocentripetal trend proposed by Fahn (1953) for the floral nectaries, i.e., evolutionary movement from the perianth towards the ovary and upward.

Seeds.—Ovule ontogeny in *Adenoa* is similar to that reported for several species of *Piriqueta* and *Turnera* (Vijayaraghavan & Kaur, 1966; Gonzalez, 2000). The episperm of *Adenoa* is reticulate; like in *Piriqueta* and *Turnera* the design of the seed coat is the result of the interaction between the large cells of the endotesta and the sclereids of the exotegmen. The episperm of *Adenoa* belongs to the striate-reticulate subtype, which is found in species of two series of *Turnera*. The epidermis of the seeds of *Adenoa* is glabrous, as in many species of *Piriqueta* and *Turnera*.

The exostome is short and rounded in the seeds of *Piriqueta*, while in *Turnera* it may be longer and occasionally have the shape of a beak elongated towards the funicle, like that observed in *Adenoa*. The chalaza is rounded in *Piriqueta* and *Adenoa*, while in *Turnera* it may be rounded or prominent and umbilicate. The aril of *Adenoa* originates at the hilum, like in *Piriqueta* and most species of *Turnera* (Arbo, 1995; Gonzalez, 2000).

Pollen.—Studies made on several species of Turneraceae (Erdtman, 1966; Melhem, 1971; Arbo, 1979; Arreguín-Sanchez et al., 1986; Arbo & Salgado, 2004) demonstrate that the pollen grains of the family are 3-colporate and prolate-spheroidal to prolate in shape. The exine is semitectate, microreticulate in *Mathurina*, *Piriqueta*, and *Turnera* (Series *Anomalae*), semitectate-reticulate angustimurate with free bacules in species of *Turnera* (Series *Turnera*) and *Tricliceras (=Wormskioldia)*. In the heterostylous species of *Turnera*, the mean size of pollen is larger in the brevistylous flowers (Arbo & Salgado, 2004). In *Erblichia* the pollen grains are reticulate angustimurate with free bacules at the lumina, whereas in *E. odorata*, the only American species, they are microreticulate; in this genus, the size of the lumina is notably smaller at the apocolpia than at the mesocolpia (Arbo, 1979).

The pollen of *Adenoa* represents a novel exine morphological type for the family: the grains are semitectate reticulate with supratectal warts scattered over the surface of the grain. However, previous investigations collectively sample a small proportion of the phylogenetic (taxonomic) diversity of the clade. Melhem (1971) described *Piriqueta* as a stenopalynous genus because palynological characters do not allow the differentiation of the species analyzed. Just a few species of *Turnera*, the largest genus, belonging to the Series *Anomalae* and *Turnera* have been described so far (Melhem, 1971; Arbo & Fernández, 1983; Rama Swamy & Bahadur, 1984, 1985; Arreguín-Sánchez et al., 1986; Arbo & Salgado, 2004). The pollen structure of most African genera remains uninvestigated. Given the relative incompleteness of comparative information on the pollen morphology of Turneraceae, it is premature to draw any systematic conclusions from this evidence.

Chromosomes.—The basic chromosome number of *Adenoa* is $x=7$, like that of *Piriqueta*, which is probably the ancestral chromosome basic number for Turneraceae. *Adenoa* is diploid, whereas in *Piriqueta* and *Turnera* polyploids are very frequent. The genus *Turnera* has $x=7$, $x=13$, and $x=5$

(Fernández, 1987; Solís Neffa & Fernández, 2000; Shore et al., 2006). According to the classification of Lima de Faría (1980) *Adenoa* has small chromosomes; their size is similar to the ones of *Turnera*, and is larger than those of *Piriqueta* (Hamel, 1965; Fernández, 1987; Lavia & Fernández, 1993; Solís Neffa & Fernández, 2000).

According to Arbo's (1995) earlier analysis of relationships among the genera of Turneraceae, the genera *Mathurina* and *Erblichia*, with free sepals and petals, are the basal taxa on the tree. All the other genera have a perianth tube, which Arbo regarded as a derived state. The American genera *Adenoa*, *Piriqueta*, and *Turnera* have a 10-nerved perianth tube and so contrast with the African genera, in which the perianth tube is 15-nerved. In *Adenoa* and *Erblichia*, with homostylous flowers, the styles diverge at the base. In *Piriqueta* and *Turnera*, which are mostly distylous, the styles are upright or excurved. *Adenoa* and *Piriqueta* have a free peduncle, while in *Turnera*, the peduncle may be free, adnate with the petiole, or absent altogether.

The above analysis of the American genera of Turneraceae suggests that *Erblichia* is

TABLE I
ANATOMICAL CHARACTERS DISTINGUISHING *ADENOA*, *PIRIQUETA*, AND *TURNERA*.

Character	*Adenoa*	*Piriqueta*	*Turnera*
Tector hairs	stellate	porrect-stellate, stellate, simple unicellular, simple pluricellular	simple unicellular, rarely pluricellular, stellate
Glandular hairs	absent	setiform, claviform, microcapitate	claviform, microcapite, sessile-capitate, stipitate-capitate
Colleter type	standard, lachrymiform, sessile	standard, lachrymiform, sessile	standard, lachrymiform, sessile, troclear
Colleter location	cotyledons, primary stem, leaves, prophylls, petals	stipules, foliar teeth, prophylls	stipules, foliar teeth, prophylls
Leaf type	xeromorphic	mesophytic	generally mesophytic, ericoid in *T. revoluta* and *T. genistoides*
Stomata location	hypostomatic	amphistomatic	amphistomatic, hypostomatic
Mesophyll	dorsiventral	dorsiventral	dorsiventral, isobilateral
Bundle sheath	parenchymatous	tanniferous	tanniferous (29 spp.), parenchymatous (8 spp.), sclerenchymatous (11 spp.)
Extra floral nectaries	absent	rarely present	often present
Flowers	axillary	axillary	axillary or epiphyllous
Adnation	perianth tube	perianth tube	perianth or floral tube
Ovules	straight micropyle	zig-zag micropyle	zig-zag micropyle
Floral nectary location	perianth tube	perianth tube, staminal filaments	perianth tube, staminal filaments, nectar pockets
Pollen exine	6 μm thick, semitectate reticulate, supratectal warts	semitectate-microreticulate	2.8–3.5 μm thick, semitectate-microreticulate, reticulate, free bacules
Seed episperm	striate-reticulate; glabrous	reticulate, exceptionally knotty; glabrous or papillose	reticulate, striate, somewhat knotty or cristate; sometimes papillose
Aril location	hilar	hilar	hilar, exceptionally rapheal
Chromosome base number	$x=7$	$x=7$	$x=7$ $x=13$ $x=5$
Chromosome number	2n=14	2n=14, 28, 42	2n=14, 28, 42, 56, 70; 2n=26; 2n=10, 20, 30, 40

distantly related to *Adenoa*, *Piriqueta*, and *Turnera*, which may form a clade (Arbo, 1995). The information presented in this work on anatomy and chromosomes corroborates this hypothesis (Table I), while the pollen exine morphology sets *Adenoa* apart. To further assess the phylogenetic position of *Adenoa*, a molecular phylogenetic study of Turneraceae is needed, which will also be important to understand character evolution within the family. So far, the only molecular phylogeny within the family Turneraceae was an analysis of 37 taxa of *Turnera* (Truyens et al., 2005).

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