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Fruit and seed morphometry and seed structure of the potentially invasive *Calotropis procera* (Aiton) W.T. Aiton (Apocynaceae)

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Abstract

Background: *Calotropis procera* is a plant native from the tropics and subtropics of Asia and Africa. In Mexico, its presence is now registered in Yucatán, Quintana Roo and Oaxaca.

Questions and / or Hypotheses: Fruit and seed morphometry vary among places where it is native or exotic? Are seed structures well identified in *Calotropis procera*?

Studied species: The exotic *Calotropis procera* has been already reported in several states in Mexico, however, information about its reproductive biology in Mexican populations is scarce.

Studied site and dates: Fruits were collected in Oaxaca, Mexico in September 2019.

Methods: We obtained fruit and seed morphometric data and seed images with microscopy.

Results: The fruits are green and ovoid. The average number of seeds per fruit is 364 ± 86 (SD). The seed is brown and flattened dorsoventrally. The length of the seeds is 8.37 ± 0.413 mm, the width is 5.01 ± 0.39 mm, and the weight is 10.5 ± 7.86 mg. The position of the hilum and the micropyle is reported and the occurrence of two types of trichomes on the testa. We suggest that the protrusion on the ventral surface of the seed is the raphe.

Conclusions: From data obtained we concluded that fruit and seed characteristics are well conserved in this species. We confirmed the position of the hilum and micropyle and reported the presence of two types of trichomes in the testa. Location of the raphe is suggested, providing information that may be useful for future taxonomic studies.

Keywords: Apocynoideae, Mexico, seed morphology, seed morphometry.

Resumen

Antecedentes: *Calotropis procera* es nativa del trópico y subtrópico de Asia y África. En México se encuentra en Yucatán, Quintana Roo y Oaxaca.

Preguntas y / o Hipótesis: ¿La morfometría de frutos y semillas varía en donde es nativa o exótica? ¿Las estructuras de la semilla de *Calotropis procera* han sido bien identificadas?

Especie de estudio: La exótica *Calotropis procera* ya se ha reportado en varios estados de México, sin embargo, hay poca información sobre su biología reproductiva en las poblaciones mexicanas.

Sitio y años de estudio: Los frutos se colectaron en Oaxaca, México en septiembre de 2019.

Métodos: Se obtuvieron datos morfométricos de frutos y semillas y se tomaron imágenes mediante microscopía.

Resultados: Los frutos son folículos ovoides y coriáceos. El promedio de semillas por fruto es de 364 ± 86 (DE). La semilla es café y aplanada dorsoventralmente. El largo es de 8.37 ± 0.413 mm, ancho de 5.01 ± 0.39 mm y el peso es de 10.5 ± 7.86 mg. Se reporta la posición del hilo y del micrópilo y la existencia de dos tipos de tricomas en la testa. Sugerimos que el relieve en la superficie ventral de la semilla es el rafe.

Conclusiones: De los datos obtenidos concluimos que las características de frutos y semillas están bien conservadas en esta especie. Confirmamos la posición del hilo y del micrópilo, y reportamos la existencia de dos tipos de tricomas en la testa. Se sugiere la posición del rafe, lo que puede apoyar en futuros estudios taxonómicos.

Palabras clave: Apocynoideae, México, morfología de semillas, morfometría de semillas

The Apocynaceae family is one of the most diverse groups of the world with over 4,000 species (Endress *et al.* 2014, 2018). Vines, bushes, trees, and herbs that grow in temperate, warm and humid forests, and xeric habitats from around the world, constitute this complex group with a complicated floral morphology and whose ecology is still poorly understood (Endress *et al.* 2018). In Mexico, this family has over 50 genera and 400 species distributed in three groups: rauvolfioids, with 11 genera and 46 species; apocynoids, with 14 genera and 57 species; and the Asclepiadoideae subfamily, the most diverse group of the family in the country with 27 genera and 315 species (Alvarado-Cárdenas *et al*. 2020).

There is extensive available information of the Apocynaceae family in flora studies, new species descriptions, taxonomic revisions and phytochemistry on some genera of general interest because of its distribution, relationship with other species and potential uses, such as *Asclepias* L. and *Calotropis* R. Br. (Alvarado-Cárdenas *et al*. 2020). Particularly, seed structure, seed anatomy and seed germination are yet to be explored in this group to understand more about its reproduction and propagation.

The genus *Calotropis* R. Br. is circumscribed in the Asclepiadoideae subfamily, inside the subtribe Asclepiadinae, and is sister to the generic complex of *Asclepias* (Goyder *et al*. 2007). The genus consists of three species: *Calotropis acia* Buch.-Ham., *Calotropis gigantea* (L.) W.T. Aiton, and the subject of this study, *Calotropis procera* (Aiton) W.T.Aiton. *C. procera* is currently distributed in the tropics and subtropics around the world (PIER 2011, USDA 2023); in its native land, it grows in deserts and sand dunes, while in the exotic areas it forms part of ruderal and xeric vegetation in arid climates. Brazil and Australia are countries that consider this species as invasive (Fabricante *et al*. 2013, Bufebo *et al.* 2016), though reasons to include it in this list have been poorly exposed (Meadley 1971, Parsons & Cuthbertson 2001).

In Mexico, according to the NaturaLista citizen science page (NaturaLista 2023), data from the National Herbarium (MEXU) and Carnevali (2010), *Calotropis procera* is now present in the states of Quintana Roo, Yucatán, Chiapas, Oaxaca and Michoacán, where a deciduous dry forest vegetation is dominant. Oaxaca is the state with the largest number of registered specimens. The National Commission for Biodiversity list of exotic invasive species maintains the status of exotic present in the country (CONABIO 2023), while the one published by the Natural Resources and Environment Secretariat does not include this species (SEMARNAT 2023).

There are several studies regarding *C. procera* with respect to pharmacology and medicinal uses (Rasik *et al*. 1999, Bhaskar & Ajay 2009, Quazi *et al*. 2013, Bairagi *et al*. 2018), a few others of invasiveness (Fabricante *et al*. 2013, Leal *et al*. 2013, Sousa-Sobrinho *et al*. 2013, Bufebo *et al.* 2016), some about germination aspects (Sen & Chatterji 1965, Labouriau & Valadares 1976, Leal *et al*. 2013, Oliveira-Bento *et al*. 2013, Galal *et al*. 2015, Taghvaei *et al*. 2015, Navarrete-Sauza & Rojas-Aréchiga 2023), taxonomy (Heneidak & Hassan 2005, Gabr 2014), and some reviews (Dhileepan 2014, Bebawi *et al*. 2015, Hassan *et al*. 2015, Kaur *et al*. 2021). Despite this amount of data, information about the seed structure is scarce and ambiguous (*i.e.,* no clear information about the position of the hilum and of the micropyle; Al Nawaihi *et al*. 2006, Gabr 2014).

Therefore, the aim of our study was to identify structural and morphometric differences in the fruits and seeds of *Calotropis procera* in Oaxaca, Mexico, compared to fruits and seeds studied elsewhere; and to identify some seed structures that have not been reported correctly that would help for future taxonomic and ecological studies.

Materials and methods

Study species. Calotropis procera (Aiton) W.T. Aiton is a shrub rarely above 6 meters tall; it has corky stems, highly branched at the base (Figure 1A). The mature leaves are almost sessile, up to 20 cm long and 15 cm wide, oblong-ovate to broadly ovate, with an apiculate apex and a narrowly cordate base, covered by a white tomentum. The inflorescences are lateral, cymose and umbellate, mostly solitary at the nodes, covered by a white, cottony tomentum. Bracts ovate-lanceolate, erect, with subacute apex. Lobes of the calyx ovate-lanceolate, with sharp points. The corolla is purple at the apex of the petals and white at the base, with the floral tube shorter than the lobes. Five coronal scales, broad, adnate to, and long as or longer than staminal column, glabrous, with slightly ciliate margins; bifid apex with-out auricles; basal spur acute, curved. Anthers tip with a thin, whitish membrane (Figure 1B). Pollinium is oblong, lanceolate, caudicular, pendulous, solitary in each locule of the anther. Dark brown corpuscle, rod-shaped formed by two cells. Style apex 4 mm long, pentagonal. Follicles inflated before forming the line of dehiscence, subglobose to obliquely ovoid, up to 13 cm long and 7 cm wide (Rahman & Wilcock 1991, Hassan *et al.* 2015) (Figure 1C). The numerous seeds are brown, ovate, and flattened, with a tuft of silky hairs. When germinating, the seedlings can grow in soils with few nutrients and under adverse weather conditions (Parsons & Cuthbertson 2001, Lottermoser 2011).

In the countries where *C. procera* is native, the uses of this and its sister species are various: the corky stem is used to obtain fibers; the tuft of silky hairs from the seeds is used to stuff pillows. Also, it has several medicinal uses, such as the use of the dried leaves for rheumatic pain and inflammation, the root extract as a tonic and the milky latex as a remedy for leprosy, as a disinfectant for open wounds, and as a toothpaste to alleviate tooth pain (Bebawi *et al.* 2015). Extracts of aerial parts and latex have insecticidal, allelopathic and antifungal properties (Begum *et al*. 2010, Abdel-Farid *et al*. 2013, Jabeen *et al*. 2013, Manrookar *et al*. 2014).

Collection sites. The information for the collection sites in the state of Oaxaca was obtained from data of the National Herbarium (MEXU) and from the coordinates provided by Dr. Leonardo Alvarado-Cárdenas. A collection route was generated in the Google Earth Pro program.

As this plant is found alongside roads and highways, the fruit collection began in the municipality of San Cristóbal, between the municipalities of Santa María Tequisistlán and Santa María Jalapa del Marqués (16° 26' 16.09" N; 95° 31' 28.13" W), continued at some points within the urban area of Tehuantepec (16° 19' 28.69 N; 95° 14' 27.72" W) and ended on the La Ventosa-Salina Cruz highway, approximately 5 km before the Tehuantepec toll booth (16° 12' 13.47" N; 95° 18' 47.44" W), in the municipality of Santo Domingo Tehuantepec. We collected 78 fruits from twenty individuals in September 2019.

Climatic information obtained from the municipality of Santo Domingo Tehuantepec reported a temperature range between 20 and 30 °C; a minimum annual precipitation of 600 mm, and a maximum of 1,000 mm; the weather type is Aw; warm subhumid with summer rains (García 1990, INEGI 2005).

Fruit and seed description. We observed and measured 71 fruits. Fruit observations were made with the naked eye, and size was determined with a digital caliper (Gimex). Photographs were taken with a Nikon D3200 professional camera (18-55 mm lens), later processed with the Adobe Photoshop program. Subsequently, the seeds were extracted from the fruits and observed with the naked eye and with a stereoscopic microscope (Leica ZOOM 2000); photographs were taken with a cellphone (Samsung Galaxy A8 2018), later processed with the Adobe Photoshop program.

Seed morphometric data were obtained from 1,450 randomly chosen seeds. The length and width were obtained with a digital caliper, and the weight was obtained using an analytical balance with a precision to ten-thousandths (Sartorius CP225D). The mean and standard deviation were obtained for each measurement.

Images with a Scanning Electron Microscopy (SEM) were obtained in two stages: firstly, 25 seeds were processed and nine of these were chosen for observation. The samples were placed in an aluminum sample holder with a conductive tape, then were dried to a critical point and covered with a thin layer of gold with a Denton Vacuum Desk II ionizer (Moorestown, New Jersey) (Meek 1977, Bozzola & Russel 1999). Subsequently, the observations were made with a JEOL JSM-5310LV microscope at 15 kV in the Scanning Electron Microscopy Laboratory of the Faculty of Sciences, National Autonomous University of Mexico (UNAM). Secondly, 30 seeds were processed and eleven were chosen for observation. The samples followed the same desiccation method but were covered with the gold layer with a Quorum Q150R ES ionizer (Sacramento, California) and observed with a Hitachi SU1510 microscope at 10 kV (Chiyoda, Japan) at the Laboratorio de Microscopía y Fotografía de la Biodiversidad 1 of the Biology Institute, UNAM.

Finally, for optic microscope images, twenty mature seeds were processed and hydrated for three days. Before fixation, a dorsal cut was made to achieve infiltration. They were fixed with FAA (formaldehyde, glacial acetic acid, 96 % ethyl alcohol, water, 5:50:10:3.5) for three days and softened with GAW (glycerine, alcohol, water, 2:1:1). They were dehydrated in a gradual ethanolic series (30, 50, 70, 85, 96 and 100 %) for 24 h in each alcohol and included in

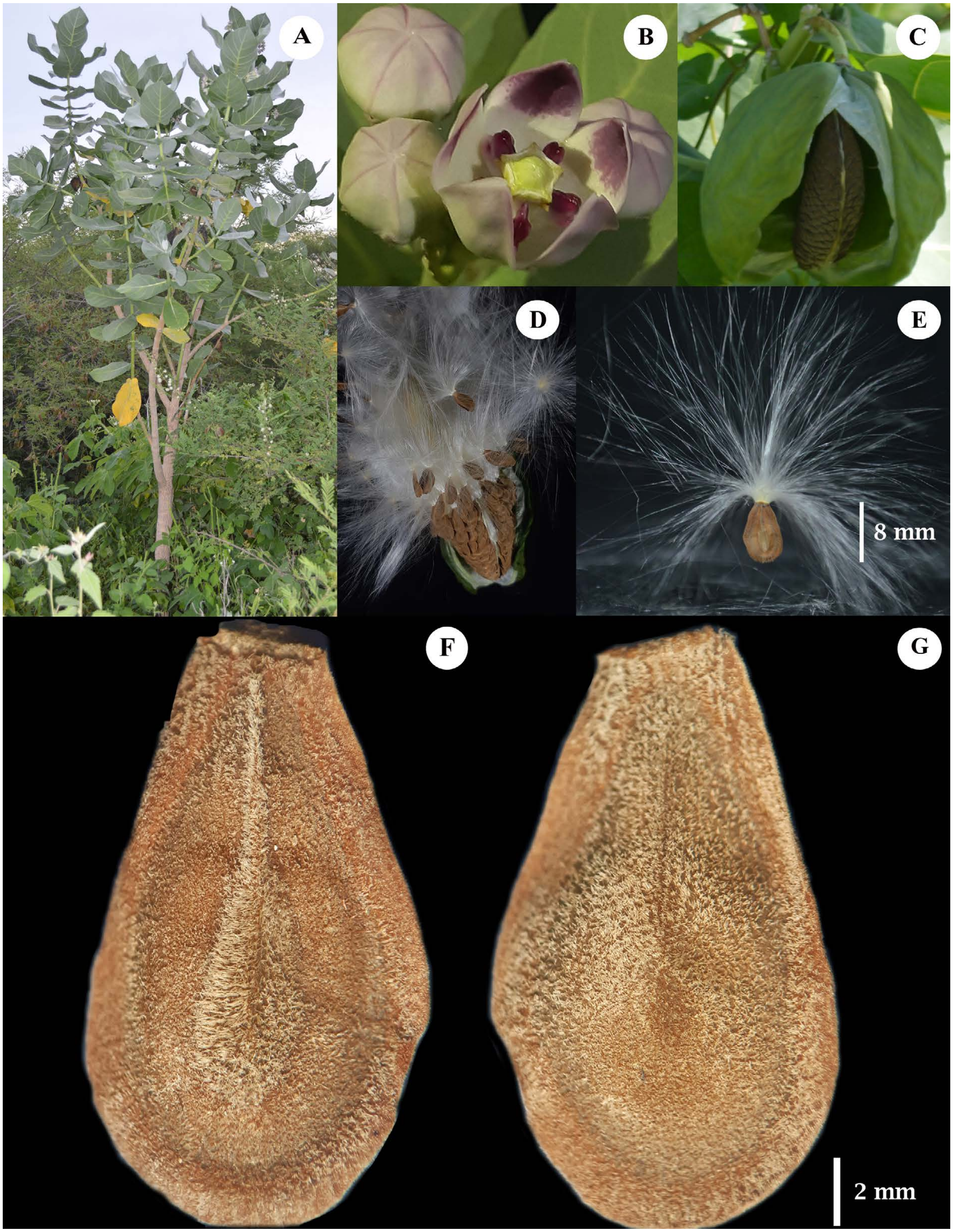

Figure 1. Vegetative and reproductive structures of *Calotropis procera* from Oaxaca, Mexico. A. View of the plant in the municipality of San Cristóbal. B. Inflorescence showing buds and flower in anthesis. C. Mature fruit attached to the mother plant. D. Mature fruit showing seeds before dispersal. E. Single seed showing the coma. F-G. Ventral and dorsal surface of the seed, respectively. Photographs: E. Navarrete-Sauza.

Paraplast Plus (Sigma-Aldrich). The material was cut in an American Optical rotation microtome, model 820 (Southbridge, Massachusetts) at 10 µm thickness and stained with safranin O (Hycel)-fast green FCF (Hycel) in methyl cellosolve (Meyer) (Márquez-Guzmán *et al*. 2016). Photomicrographs were taken on a Leica DM750 Microscope with integrated Leica ICC50 HD camera (Wetzlar, Germany).

Histochemical procedures. Seeds of *C. procera* were subjected to the following histochemical tests: Periodic Acid-Schiff (PAS) for non-soluble polysaccharides and naphthol blue black OH 96 % to show up proteins (Márquez-Guzmán *et al*. 2016).

Periodic Acid-Schiff (PAS).- Cuttings were deparaffinated in the stove at 56 °C and rehydrated in a gradual ethanol series up to water. Periodic acid was applied for 15 minutes, then washed with distilled water and applied Schiff reactive for 15 minutes, then washed with distilled water and then acetic acid 2 % for 1 minute was applied, then washed with distilled water and dehydrated up to xylene and were mounted in a synthetic resin for observation.

Starch and some wall polysaccharides, as well as some phenolic compounds stain to red or magenta in a positive reaction (Ruzin 1999, Márquez-Guzmán *et al*. 2016).

Naphthol blue black.- Cuttings were deparaffinated in the stove at 56 °C and rehydrated in a descending gradual ethanol series up to ethanol 50 % and a few drops of naphthol blue black were applied for 5 minutes, then washed with butanol and put into xylene for 3 minutes. Then were mounted in a synthetic resin for observation. Proteins stain in blue for positive reactions (Márquez-Guzmán *et al*. 2016).

Results

Fruit description. The fruits are green, ovoid-shaped, leathery follicles, which are swollen when immature. They lose volume as the entire follicle dries progressively and the dehiscence line becomes noticeable (Figure 1C). Fruit maturation time was not recorded in the collection site, but under laboratory conditions the immature fruits ripened and dehydrated in no more than two weeks. When fruits were completely mature, they opened widely along the dehiscence line, contracted and reverted to expose the brown seeds (Figure 1D). Of the 78 fruits collected, 29 of them were fully mature.

We registered the length of 71 fruits, which allowed us to estimate the change in size due to dehydration. When the fruit is not yet fully mature, it measures approximately 13 cm long and when it dries, its size is reduced to approximately 6 cm long. The average number of seeds per fruit was 364 ± 86 (SD); the maximum number of seeds in a fruit was 481, and the minimum was 95. We obtained a total of 28,401 seeds. Seed description was made from the 29 mature fruits at collection from which 1,450 seeds were randomly observed, measured, and weighed.

Seed description. Calotropis procera seeds are brown in color (7505c, PANTONE® 2019); dorsoventrally flattened and with an obovate shape. The seed coat shows an extension called wing that surrounds almost completely the seed, except in the hilum region where the silky hair appendages develop, collectively called a coma (Figure 1E). This structure is associated with their most common dispersal mode: anemochory. They have two surfaces that from now on will be identified as ventral; the surface that was in contact with the placenta and attached to the fruit (Figure 1F); and dorsal, which is opposite to the ventral surface and can be observed when the fruit opens, and the seeds are still attached to the placenta (Figure 1G). Morphometric data obtained from the seeds (N = 1,450) are shown in Table 1.

Table 1. Morphometric data of length, width, and weight of *Calotropis procera* (Aiton) W.T. Aiton. seeds (N = 1,450), where Max = maximum; Min. = minimum; SD = Standard Deviation.

Length (mm)			Width (mm)			Weight (mg)		
Max.	**Min.**	**Mean (±SD)**	**Max.**	**Min.**	**Mean (±SD)**	**Max.**	**Min.**	**Mean (±SD)**
9.36	6.06	8.37 ± 0.41	7.98	2.82	5.01 ± 0.39	12.39	1.92	10.5 ± 7.86

On both surfaces of the seed, an indument gives it a velvety appearance due to trichomes occurrence. Under the stereoscopic microscope, on the ventral surface, we noticed that the number of trichomes increases -and the color of the seed coat is lighter- in the wing and from the center of the seed to the area where the funiculus is located, forming a racket-shaped figure (Figure 1F). The ventral surface is often slightly concave, and it is possible to distinguish the wing and part of the funiculus. On the dorsal surface, regularly convex, the only structure that can be identified is the wing, and when seen under the stereoscopic microscope it shows a relatively homogeneous color, due to the distribution and amount of trichomes in the seed surface.

There are multiple trichomes of many sizes over the seed surface (Figure 2A). The seed showed two types of ornamentation: the first one consisting of short, rounded, papillae-shaped trichomes (glandular trichomes, Wagner *et al*. 2004, Figure 2B, C). and the second one of long, ensiform projections (simple trichomes, Wagner *et al*. 2004, Figure 2D). With the histochemical techniques used, glandular trichomes gave positive for PAS and for Naphtol blue black, meaning that glandular trichomes contain, and possibly produce, polysaccharides and proteins (Figure 2B, C) that are potentially secreted by these structures, found all over the seed.

The simple trichomes are numerous in the wing and in the ventral surface of the seed (Figure 3A). Particularly where the raphe is located, these trichomes are considerably longer and agglomerated (Figure 3B); in the middle area of the seed, the distribution of trichomes is relatively homogeneous (Figure 3C) whereas those on the wing are shorter, but also agglomerated (Figure 3D). The length of both types of trichomes is shown on Table 2. The glandular trichomes are found on both surfaces of the seed and show no apparent pattern in terms of quantity and size and have a homogeneous length along the seed.

A transversal image of the seed taken under light microscope clearly showed the testa, cotyledons, apical meristem, hypocotyl, radicle and funiculus (Figure 4A). Transversal and horizontal SEM images (Figure 4B-D) allowed distinguishing the funiculus and its vascular tissue. A structure observable to the naked eye and not previously described for this species is the raphe. The identification of the hilum allowed us to observe a protrusion that is below the seminal cover and that runs through a continuous line in the ventral surface of the seed from the vascular bundles of the funiculus (Figure 4D) to the centre of the seed (Figure 4E), going through the racket-shaped area, which can be identified because of the lighter color from trichomes arrangement with respect to the rest of the ventral surface. Although, to confirm this finding, more morpho-anatomical studies are required.

A SEM image of a germinating seed of *C. procera* exhibited the radicle emerging from the micropyle (Figure 5A) and a longitudinal section of the germinating seed showed the complete radicle, hypocotyl as well as the testa and cotyledons (Figure 5B). The funiculus in *C. procera* seeds is broken when the seed is separated from the fruit, remaining only as a structure inside the hilar region located on the ventral surface of the seed, near the place where the coma develops and where the coma scars are observed after their detachment (Figure 5C, D). Also, in this region the micropyle can be noticed, though difficult to distinguish because it is obliterated.

Discussion

Fruit description. The information found in the available literature on the fruit description of *Calotropis procera* is scarce. Most works only briefly mention the type of fruit (Heneidak & Hassan 2005, Sousa-Sobrinho *et al.* 2013), other comment its shape and occasionally its size, weight and number of seeds per fruit (see Table 3). Likewise, Hassan *et al*. (2015) mention that the fruit opens completely and inverts when it matures, exposing the seeds to be dispersed. The morphometric data previously reported mostly agree with the results obtained in this work and with the description of Hassan *et al*. (2015) regarding the drying process of the fruit and seeds exposure before dispersal.

Seed description. Concerning about the seed length and width, some works mention that the length varies from 5.5 to 8 mm and that the width ranges from 4.2 to 6 mm (see Table 3). In this work the length variation goes from 6.06 to 9.36 mm and the width variation from 2.82 to 7.98 mm with an average data of 8.37 ± 0.413 mm long and 5.01 ± 0.39 mm wide; Table 1). Results are similar comparing the ones obtained here with other works, independently if the work was done where *C. procera* is a native or an exotic species.

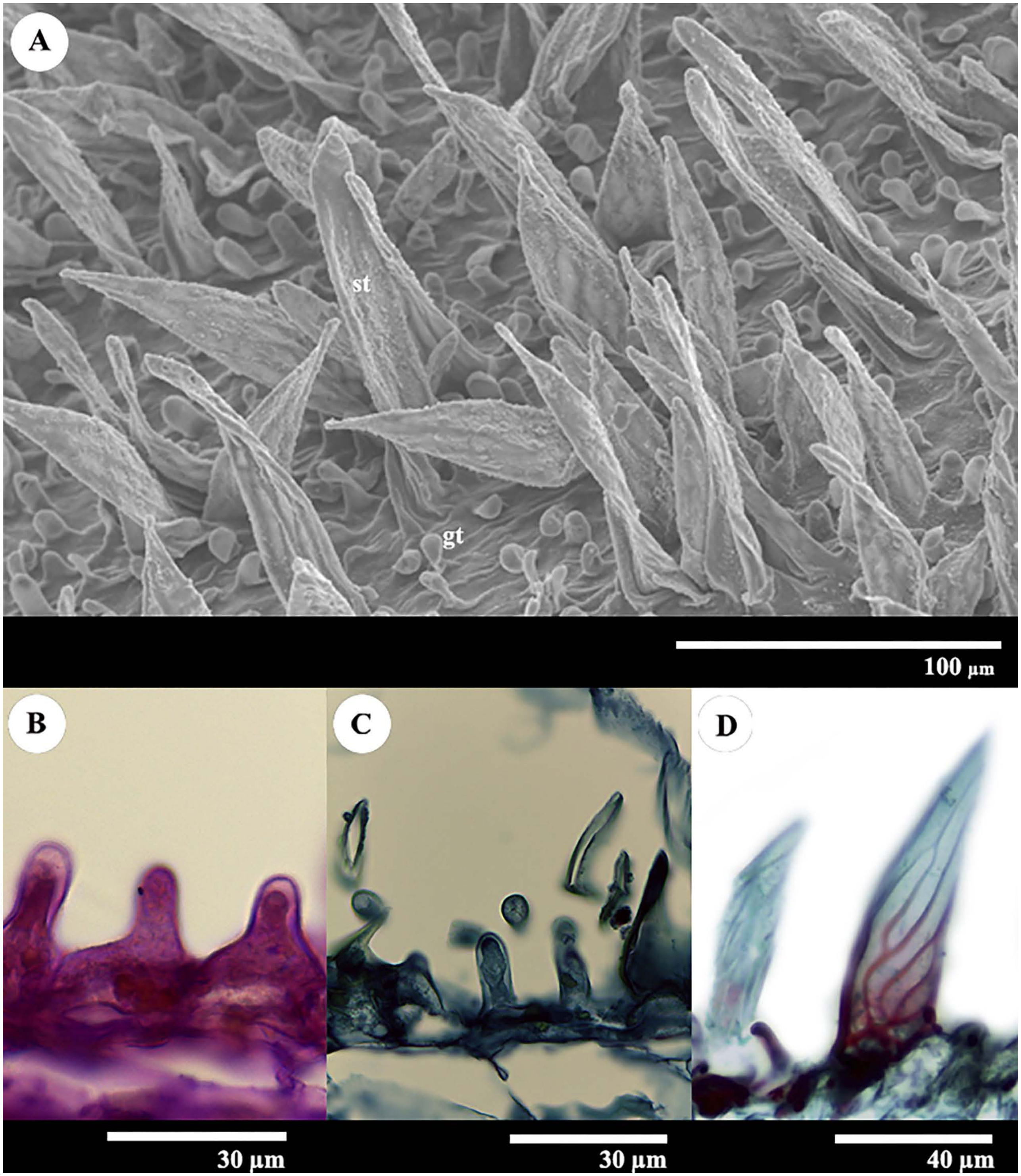

Figure 2. SEM image and light microscope images of a *Calotropis procera* seed showing: A. Simple and glandular trichomes (st, gt respectively), B. Glandular trichomes stained with PAS, C. Glandular trichomes stained with Naphtol blue black and, D. Simple trichomes. Photographs: M.B. Mendoza Garfias (A), M.K. Pérez-Pacheco (B, C, D).

Table 2. Mean length (M) and maximum (Max) and minimum (min) length in micras (µ) of simple and glandular trichomes on three different areas on the seed of *Calotropis procera* (N = 30 for each area).

Types of trichomes	Wing			Raphe			Middle		
	Ventral /dorsal zone (µm)			Ventral zone (µm)			Ventral /dorsal zone (µm)		
	M±SD	Max	min	M±SD	Max	min	M±SD	Max	min
Simple	49.9 ± 12.79 / 57.76 ± 15.42	75/88	26/37	115 ± 19.5	151	58	79.5 ± 27.35 / 95.7 ± 27.29	144/151	32/56
Glandular	11.8 ± 2.23 / 13.66 ± 2.38	9/16	9/14	14.23 ± 3.44	23	7	12.3 ± 1.53 / 11.5 ± 2.11	8/16	6/16

Seed weight of *C. procera* has been poorly reported. It is very likely that the seed weight of this species was firstly reported by Sen & Chatterji (1965) who obtained a minimum average weight of 0.568 g and a maximum of 0.98 g in 100 seeds. These data agree with the results reported here, where the average weight obtained is 10.5 mg ± 7.86 SD (Table 1). Other studies have reported seed weight, too (see Table 3). Seed mass reported here (Table 1) was at least five times lower than the dry seeds of Leal *et al*. (2013). Oliveira-Bento *et al*. (2013) obtained the average weight of a single seed by weighing 1,000 seeds and calculated that each seed weighed 8.54 mg. Differences reported in these studies and here with respect to weight could be due to the different weighting methods and instruments used for its determination, to a maternal effect induced by the environmental conditions at the time of seed development or to the degree of maturation or desiccation of collected seeds, among others.

The number of seeds per fruit is an important plant attribute because it represents the potential number of individuals that may establish in a certain area (Herrera 1991). In the present work, we obtained an average of 364 ± 86 SD seeds per fruit (Table 1), which agrees with other investigations (see Table 3). So, it is possible to conclude that *C. procera* can produce more than 300 seeds per fruit.

With respect to fruit and seed characteristics obtained for *C. procera* from plants either from where it is a native species or an exotic one, we did not find differences. Regarding fruit size, the variation reported could be due to fruits collected at different stages of maturation-desiccation as we noticed at the time of harvest. Fruits already full ripen rapidly desiccate and reduce their size. As for the seed size, it does not show a high variation taking into consideration the minimum and maximum values reported in this and other studies. As we already mentioned above, the higher variation found in seed weight may be probably due to different methodological procedures not reported.

Regarding the identification of the external structures of the seeds, description of trichomes is scarcely reported. Most of the literature focuses on vegetative parts, including flowers (Meira *et al*. 2014, Medina *et al*. 2021, Watts & Kariyat 2021); however, functions and shapes of these structures are very similar despite its location. For example, simple trichomes are reported to confer, among many other functions, protection to the seed from excessive UV light and water retention (Wagner *et al*. 2004, Watts & Kariyat 2021). This is very important for species that grow in arid environments, such as *C. procera*, because UV radiation can damage the embryo enough to inhibit germination. Another potential functions of trichomes in the seed is their anchorage to the soil which may facilitate germination process (Wagner *et al*. 2004). In the case of the glandular trichomes, it has been reported that the potential functions are mostly associated to protection (Wagner *et al*. 2004). In seeds, secretions of glandular trichomes could even immunize seedling for its protection in establishment (Wagner *et al.* 2004). For *C. procera*, neither of these trichomes have been reported; Gabr (2014) described the texture of the seed as hairy with papillary coat, but any description was made.

According to the positive reaction obtained for polysaccharides (PAS) and proteins (Naphtol blue black), glandular trichomes may have a secretory function probably for protection due to the nature of these compounds reported in other structures and species, such as alkaloids, terpenes, phenolics and other substances (Levin 1973), that are still to be studied for this species.

About other observations about the external structures, since the connection between the funiculus and the seed is observed, the structure that can be identified in that area corresponds to the hilum. This structure has been wrongly described as an oblong-ovate structure occurring in the central position in the seed (Gabr 2014) and in a study con-

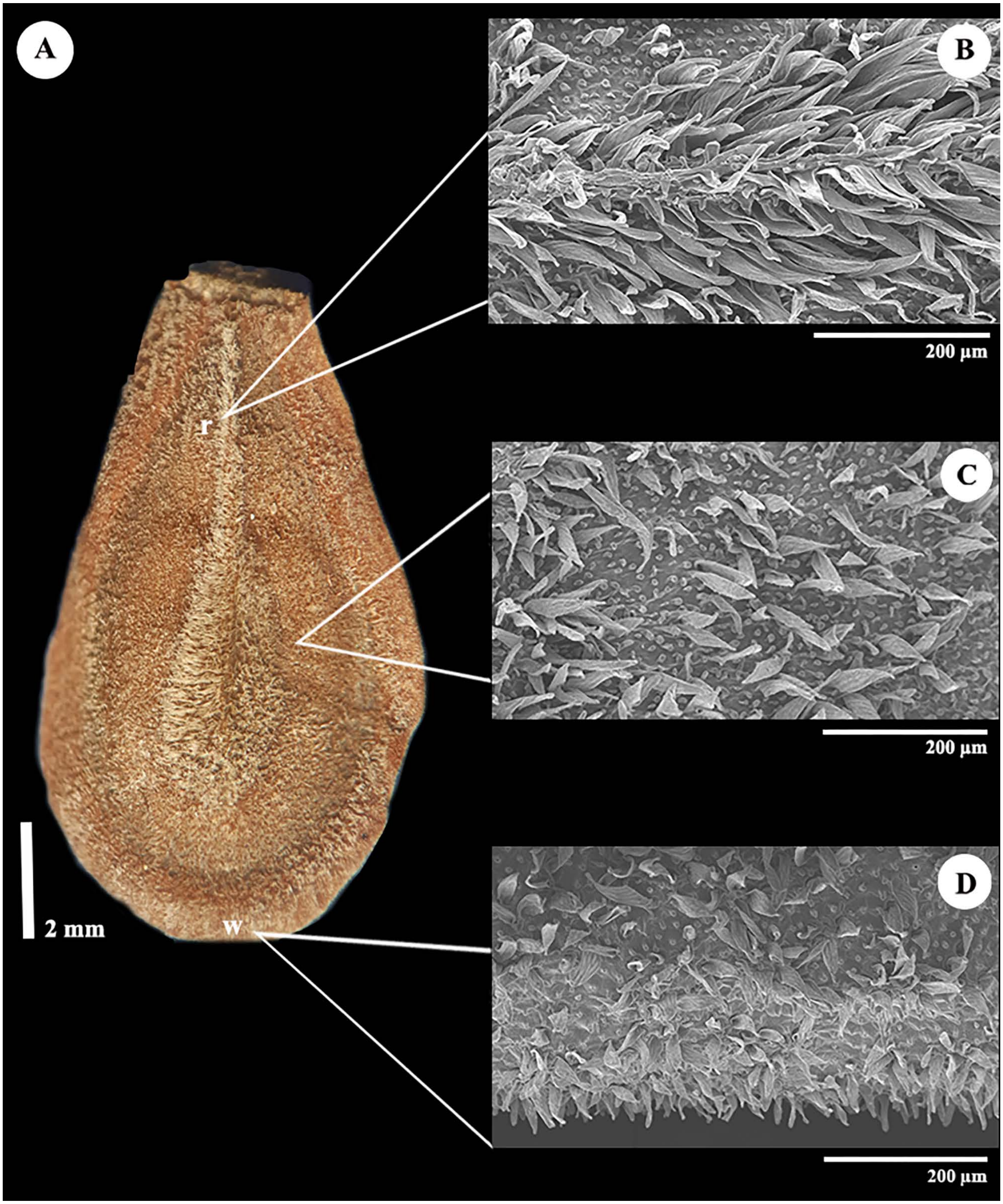

Figure 3. SEM and light microscope images of a *Calotropis procera* seed showing: A. Ventral surface of the seed, B. Magnification of the raphe showing simple trichomes and, C. Magnification at the middle area of the seed showing simple and glandular trichomes, D. Magnification of the seed wing showing simple trichomes. Photographs: E. Navarrete (A), M.B. Mendoza Garfias (B, C, D).

Table 3. Fruit and seed characteristics of *Calotropis procera* reported in revised literature and this study. The * indicates where it is an exotic species. Dash means no data.

Reference	Structure	Appeareance	Length x width	Weight	Number of seeds per fruit
Rahman & Wilcock 1991	Fruit	Follicles inflated, subglobose to obliquely ovoid	6-12.5 × 3.7cm	-	-
	Seed	Ovate with silky hairs	-		
Heneidak & Hassan 2005	Fruit	Follicle	-	-	-
	Seed	Winged, ovate, dark brown, tomentose	7 × 5 mm		
Al Nawaihi *et al*. 2006	Fruit	Spongy follicle	-	-	-
	Seed	Ovate, hairy	6-8 × 5-6 mm		
Oliveira-Bento *et al*. 2013 *	Fruit	-	7.41 × 6.79 cm	23.53 g	245-476
	Seed	-	0.55 × 0.10 cm	8.54 mg	
Leal *et al*. 2013 *	Fruit	-	-		213-391 (Caatinga)
	Seed	-	6.3 ± 0.3 × 4.2 ± 0.2 mm	5.64 mg	162-403 (Restinga)
Gabr 2014	Fruit	-	-	-	-
	Seed	Brown, ovate, hairy, flattened, winged	-		
Hassan *et al*. 2015	Fruit	Green, smooth follicle subglobose to obliquely ovoid	8-14 × 6-9 cm	-	350-500
	Seed	Flat, obovate with silky pappus	6 × 5 mm		
Galal *et al*. 2015	Fruit	-	-	-	410
	Seed	-	-	-	
Parihar & Balekar 2016	Fruit	Inflated follicle, subglobose to obliquely ovoid	-	-	Large amount
	Seed	Flat, obovate, with pappus			
Al Sulaibi *et al*. 2020	Fruit	Grey green, ellipsoid or ovoid	8 - 12 cm length	-	350-500
	Seed	Brown	2.5 - 3.5 cm length (including coma)	-	
This work *	Fruit	Leathery follicles, green, ovoid	13 cm length	-	364 ± 86
	Seed	Brownish, obovate, dorsoventrally flattened	8.37 ± 0.413 mm × 5.01 ± 0.39 mm	10.5 ±7.86 mg	

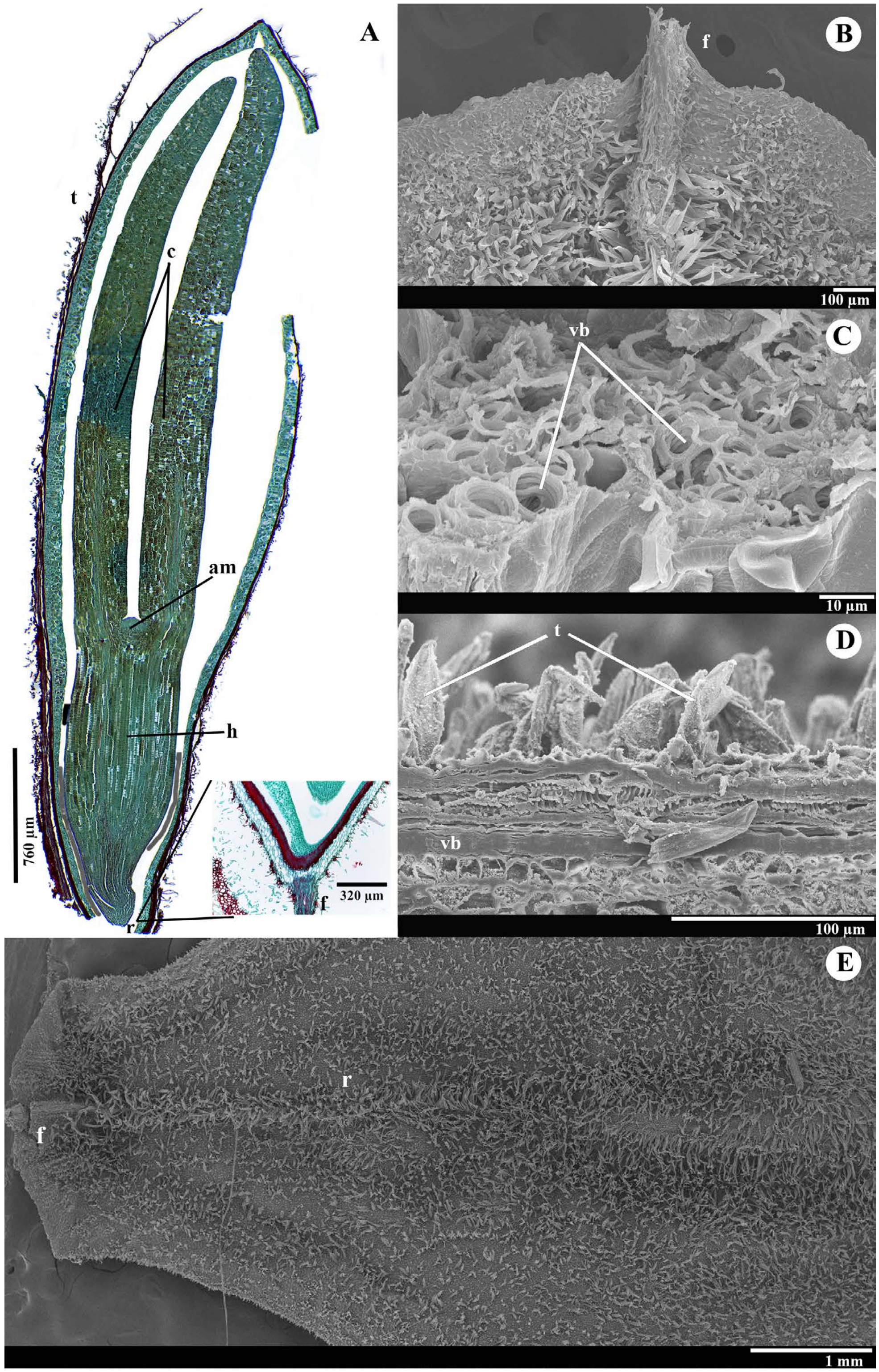

Figure 4. SEM and light microscope images of a *Calotropis procera* seed where in: A. Transversal section of the seed showing the testa (t), cotyledons (c), apical meristem (am), hypocotyl (h) and radicle (r). In the inferior section the funiculus (f) is shown. B. Transversal section of the funiculus (f), C. Vascular bundles (vb) of the funiculus. D. Longitudinal section showing the vascular bundles and simple trichomes (t). E. Ventral surface of the seed showing the raphe (r) and the funiculus (f). Photographs: M.K. Pérez-Pacheco (A), S. Espinosa Matías (B, C, E), M. B. Mendoza Garfias (D).

ducted by Ilçim *et al*. (2010) about exomorphic structures of the genus *Vincetoxicum*, a closely related genus to *Calotropis* (Potgieter & Albert 2001), the hilum was located in the ventral surface of the seed; even though Heneidak & Hassan (2005) had mentioned the position of the hilum at the narrow end of the seed under the coma. However, none of these articles showed evidence to support the existence of the hilum in the proposed area. This ambiguity resulted in difficulties for us to locate the structure and led us to rethink the position of the hilum in this species given that the seeds come from anatropous ovules (Maheswari-Devi 1964) and that the funiculus sometimes persists when the seed detaches from the placenta. In the present work, the vascular bundles can be observed within the fracture zone of the funiculus (see Figure 3C), so the hilum must be found in the region where the funiculus detaches completely. Also,

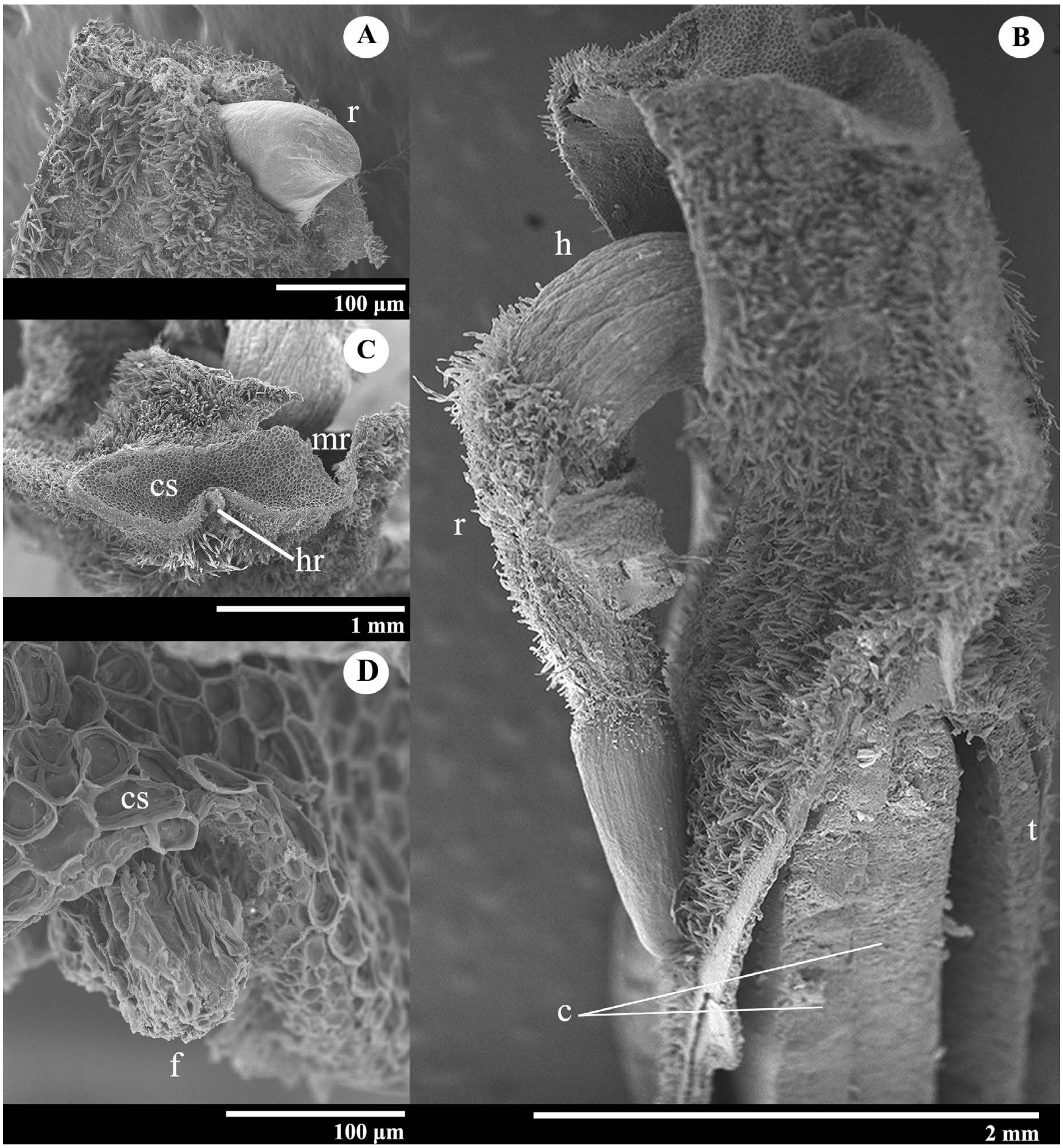

Figure 5. SEM images of a *Calotropis procera* seed. A. Emerging radicle (r) through the micropyle, B. Longitudinal section of a germinated seed showing complete radicle (r), hypocotyl (h), cotyledons (c), micropyle (m) and testa (t), C. Hilar region (hr), micropylar region (mr) and coma scars (cs), D. Close-up of the hilar region showing the funiculus (f) and the coma scars (cs). Photographs: M. B. Mendoza Garfias (A), S. Espinosa Matías (B, C, D).

we propose the position of the micropyle in this region because in most cases the radicle emerges from it as seen in figure 4B, though in this species we observed that germination occasionally occurred laterally, causing a horizontal split of the seed separating the ventral from the dorsal surface which probably may occur when the micropyle is completely obliterated and the radicle finds no way out. This may imply that *C. procera* seeds can imbibe water for germination from other parts of the seed rather than only by the micropyle, but still needs to be confirmed.

In the ventral surface of the seed, the protrusion that we consider the raphe is yet to be confirmed, but possible by definition (Font-Quer 2001). This affirmation comes from the fact that vascular bundles run from the funicle to at least the center of the seed (Figure 4) creating a conspicuous bulge easily identified at the naked eye because of the numerous long simple trichomes that form a line in the middle of the surface of the seed.

In the region where the funiculus and the raphe are described, SEM photographs show a noticeable difference between the tissue from which the coma emerges, in the region of the polygonal scars, from the surrounding tissue, which indicates a different origin from that of the micropyle (Figure 5D). This is in accordance with the embryological study done by Maheswari-Devi (1964), who reported that the coma in *C. gigantea* starts its development right after fertilization and that the epidermal cells of the integument in front of the funiculus initiate their activation to finally develop the silky hairs. Some taxonomic descriptions of subfamily Asclepiadoideae have mentioned that the wing of the seeds surrounds the entire propagule except in the micropylar region (Endress *et al.* 2014, 2018), where the SEM photograph magnification was made.

The information of some fruit and seed characteristics that are exposed in this work, such as the number of seeds per fruit, the size of the seeds and the dispersal mode, is of great value for the study of potentially invasive species (Thomson 2001). As mentioned above, the number of seeds per fruit can be related to the number of individuals with the potential to establish themselves under adequate conditions (Herrera 1991) and we found in this research that *C. procera* produces many seeds per fruit. The variation found in the seed size could be due to the phenotypic plasticity that has been attributed to invasive species if that attribute is part of the genetic possibilities for expression under different conditions. In addition, this characteristic can be related to the size of the embryo and the future establishment of the individuals (Maron *et al.* 2004).

The coma in this species allows the dispersal by the wind which enables seeds to be easily lifted with the slightest air stream and be transported over long distances (Sharma *et al*. 2010, Menge *et al.* 2016, personal observations). Dispersal mode is a very important characteristic for the colonization of new territories and for the population dynamics, for it defines the potential area where offspring may establish (Pergl *et al.* 2011).

Since *C. procera* has become an invasive species in several countries and now is naturalized in Mexico, we consider important to generate knowledge of this species seeds to encourage future studies on ecology, seed physiology and taxonomy, among others, thus providing a better understanding of this species in its colonized areas. Likewise, the hilum, the micropyle, the raphe and the trichomes here described may be used as important taxonomic traits that together with other structures may help to differentiate groups, for example, at a subtribe level due to the similitudes in seed structure with closely related genera, such as *Vincetoxicum.* Further ontogenetic studies would provide a better understanding of the studied structures.

Calotropis procera regularly produces more than 300 seeds per fruit that are mostly dispersed by wind, providing a better chance to colonize a greater area. Morphometric data obtained coincides with that from references, pointing out that fruit and seed biometric characteristics are well conserved in this species in places where it is either a native or an exotic species. As we reviewed the literature available for the family, we could notice that the seed structures in the Apocynaceae are not well described. This represents a very important gap of information in one of the most diverse groups of plants in the world and an invitation to understand more about its reproduction and ecology.

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