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Morphological characterization of fruit, seeds and seedlings of white-seal (*Chrysophyllum rufum* Mart. -Sapotaceae)

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Abstract: *Chrysophyllum* is the second largest genus of Sapotaceae, with 81 species distributed in the neotropics. Little data are found in the literature regarding the morphology of seedlings and the early development of this genus. This study aims to morphologically characterize the fruit, seeds and seedlings of *Chrysophyllum rufum* Mart. Fruits were collected from individuals present in two fragments of the Atlantic Forest, Pernambuco. A sample of 100 seeds and 100 fruits was randomly selected to obtain the morphological data. The seeds were sown in plastic trays in a greenhouse. The fruits are bacoid, obovoid and globose with one or two functional seeds per fruit. The seeds are obovate, with the shape of the hilum ranging from elliptical transverse to oblong transverse. The embryo is cotyledonar, with a spatulated form. The cotyledons are foliaceous and whitish-translucent. The endosperm is abundant and whitish. Germination is epigeal, phanerocotylar and unipolar. The seedling has different characteristics than those of the adult individual, such as the shape and leaf consistency, type of leaf margin, type of venation variation, number of pairs of secondary veins, trichome coloring and abundance of latex.

Keywords: *morphological description, germination, propagules, Atlantic Rainforest.*

Caracterização morfológica do fruto, sementes e morfofunção de plântula do lacre-branco (*Chrysophyllum rufum* Matius - Sapotaceae).

***Resumo*:** *Chrysophyllum* é o segundo maior gênero de Sapotaceae, com 81 espécies distribuídas nos neotrópicos. Dados encontrados na literatura sobre a morfologia dos propágulos e desenvolvimento inicial do gênero ainda são escassos. O presente estudo tem como objetivo caracterizar morfologicamente o fruto, a semente e a plântula de *Chrysophyllum rufum* Mart.. Os frutos foram coletados de indivíduos presentes em fragmentos de floresta Atlântica, Pernambuco. Uma amostra de 100 frutos e 100 sementes foi selecionada aleatoriamente para obtenção dos dados morfológicos. As sementes foram semeadas em bandejas plásticas, em casa de vegetação. Os frutos são do tipo bacóide, obovóides a globosos, com uma ou duas sementes funcionais por fruto. As sementes são obovadas, hilo com forma variando de transversal elíptica a transversal oblonga. O embrião é cotiledonar, com forma espatulada. Os cotilédones são foliáceos, esbranquiçado-translúcido. O endosperma é abundante e esbranquiçado. A germinação é epígea, fanerocotiledonar e unipolar. A plântula apresenta caracteres diferentes do indivíduo adulto, tais como a forma e consistência foliar, tipo de margem foliar, variação do tipo de venação, número de pares de veias secundárias, coloração do tricoma e abundância do látex.

Palavras-chave: *descrição morfológica, germinação, própagulos, floresta Atlântica.*

Introduction

Chrysophyllum is the second largest genus, in number of species, of the Sapotaceae family, with 81 species distributed through the neotropics (Pennington 1990). The genus is represented in Brazil by 31 species, including nine subspecies, distributed along the national territory in different phytogeographic domains (Pennington 1990, Carneiro et al. 2015). The taxonomy of the genus appears to be quite undefined and complex, with species characterized by difficult morphological delimitation. Recent studies show that *Chrysophyllum* does not constitute a monophyletic group either in relation to the generic concept nor in the sections proposed in the last revision of the family (Pennigton 1990, Swenson & Andeberg 2005, Swenson et al. 2008). In accordance with this, interest in studying the morphology of the fruit, seed and seedling of *Chrysophyllum rufum* Mart. has arisen. This species, commonly known as white-seal in the state of Pernambuco, Brazil, has shrubs or trees from 8 to 20 m, with small flowers (2-3 mm), green sepals covered by dense golden-rusty trichomes, and delicate, cream-green petals. According

to the National Environmental Council (Conama 2011), *C. rufum* is an indicator species for different successional stages of the vegetation in the Atlantic Forest and is primarily found in the middle and advanced stages of regeneration. Only in the Pernambuco area does *C. rufum* occur in the initial stages of regeneration (Conama 2011). In small patches of the Atlantic Forest in Pernambuco, the remaining individuals of *C. rufum* are frequently found as part of regrowth, a phenomenon deserving of more attention by scientific research: human pressure in different Brazilian ecosystems, combined with a lack of data about the plant's conservation status, are currently serious obstacles to maintaining the species. In Brazil, *C. rufum* occurs in the northeastern (Alagoas, Bahia, Ceará, Paraíba, Pernambuco, and Sergipe) and southeastern (Espírito Santo, Minas Gerais, and Rio de Janeiro) regions, at the phytogeographic Caatinga dominium, Cerrado and the Atlantic Forest (Pennington 1990, Carneiro et al. 2015). Despite being a well collected species, little data are available in the literature regarding the morphology of seedlings and the early development of the plants. Thus, this study proposes a morphological characterization of the fruit, seed and seedling of *C. rufum* (white-seal), with consideration that studies following this approach may, besides highlighting characteristics of taxonomic value, serve as support for ecological research related to fauna and flora interactions and the recognition of species seedlings in studies of banked seed and seed rain, among others.

Material and Methods

1. Local collection

The fruits of *C. rufum* were collected from three individuals present in two fragments of the Atlantic Forest. The first fragment is popularly known as "mata do frio" and is located in the municipality of Paulista, which encompasses Parque Natural Municipal do Frio (Natural Park), the first Conservation Unit of this category in the region. The second fragment is the Ecological Park São José, located at the municipality of Igarassu/Pernambuco (7º40'21.25"– 7º55'50.92"S e 34º54'14.25"–35º05'21.08"W); it is one of the largest reserves of the Atlantic Forest in the Brazilian Northeast and property of the Usina São José (USJ).

2. Collection and processing of plant material

Mature fruits were collected directly from trees or on the ground and stored in paper bags. After collection, they were transported to the Laboratory of Floristic Coastal Ecosystems (Laboratório de Florística de Ecossistemas Costeiros - LAFLEC) at the Universidade Federal Rural de Pernambuco (UFRPE), where they were selected properly (discarding immature fruits and/or those with injury/damage by animals), described and photographed. In the laboratory, 100 fruits and 100 seeds were analyzed. The fruits were analyzed in relation to color, brightness, hairiness, dehiscence, shape, form, size (length x width), consistency and texture of pericarp and number of seeds per fruit. The seeds were observed for size (length x width), shape, color, brightness and hairiness of tegument, shape and heel position, and embryo characteristics. The terminology used to describe fruits and seeds follows the guidelines of Barroso et al. (1999). After processing the fruits and obtaining the seeds, seeds were sown in plastic trays (28 × 15 × 8 cm) in a greenhouse. As substrate for germination, we used the soil collected from the forest fragments where the individuals of *C. rufum* were found. From the pool of germinated individuals, the most vigorous individuals were selected to carry out the morphological description of seedlings, these being described using the elements suggested by Roderjan (1983): hypocotyl, cotyledons, epicotyl, eophyll (protophyll) and root. The terminology employed was based on Radford et al. (1974), Duke & Polhill (1981), Oliveira (1993) and Camargo et al. (2008). It is important to mention that the seeds presented a type of dormancy, shown by slow and discontinuous germination and a reduced number of germinated seeds.

Results and Discussion

1. Morphological description of fruits and seeds

The studied species presents bacoid fruits, light green when immature, which turn brownish-red at later stages and purple when ripe (Figure 1). The fruit originates from a superior ovary, typically six-locular and in rare cases seven-locular, with one ovule per locule. However, not all ovules complete their development. The shape of the fruit varies from obovoid to globose with a round apex and acute-obtuse base, and presents a depression in the base due to the seed shape (Figure 1). The size of the fruits vary from 9.35 to 14.25 mm long and from 8.34 to 12.83 mm wide, with one or two well developed seeds. It is important to note that few fruits present two well developed seeds with one apparently viable embryo (Figure 1). However, five to six small and non-functional seeds (Figure 1) can be found inside the fruit. Six to seven persistent and pubescent sepals are observed at the fruit base, with pedicels 5.34 to 6.79 mm long and with pubescent sepals presenting malpighiaceous ferruginous trichomes. The epicarp is smooth (though the voucher specimen has a slightly wrinkled appearance), glossy, thin, and glabrous or with thin sparse trichomes. At the apex of the fruit, one observes the persistent stigma forming a small apical (hairy apiculture). The mesocarp is sulcused and soft but slightly fleshy and whitish-purple, without a discernible odor. The endocarp is very thin, membranous, whitish, and not adhered to the seed. The propagule's characteristics may contain elements that define and separate the different taxa. Almeida Jr. et al. (2010) highlight the importance of obtaining characteristics of fruits, seeds and seedlings to support studies on Sapotaceae, helping with the species identification. In addition, studies that demonstrate the morphology of fruits and seeds of native plant species can be of great use in the production of seedlings for forest nurseries, subsidizing ecological research and forest regeneration after anthropogenic disturbances. The seed has dimensions of 9.8 to 11.95 mm in length and 7.06 to 9.9 mm in width, occupying almost the entire internal cavity of the fruit. Only one seed develops completely inside the fruit (Figure 1). The seed shape is obovate, with a smooth brow, glossy, stiff, and glabrous, with color ranging from cream to light brown (sometimes bi-color). The hilum is evident, wide, depressed, and base-ventral, with its shape ranging from elliptical transverse to oblong transverse. Around the hilum, depressions corresponding to "aborted" seeds that had not finished their development could be observed. Contour lines are observed leading down from the seed apex to the base (around the hilum). The embryo is cotyledonar with a spatulated form, perfectly distinguishing between the hypocotyl-root axis and the cotyledons, which are whitish-translucent, with two oblong, planar and membranous embryonic leaves. The endosperm surrounding the embryo is abundant and whitish in color. The cotyledons are thin and foliaceous (Figure 1). Pennington (1990, 1991) emphasized the importance of seed characteristics to species separation, especially the shape (if laterally compressed or not, the size and shape of hilum, and the presence or absence of endosperm as well as its abundance in the seed). In addition, features such as these, in conjunction with other morphological data, have provided support for the author to subdivide *Chrysophyllum* into five sections. It is also worth noting that the author describes the existence of only one seed per fruit, but the present study verifies that eventually, it is possible to find more than one well developed and viable seed.

2. Morphological description of seedling and morfofunction

Germination began 20 – 34 after sowing, with radicle protusion and subsequent formation of the hypocotylar strap (Figure 2). The seedling has epigeal germination, phanerocotylar and unipolar, with axis positioned between the cotyledons. The hypocotyl is slightly elongated (1.5 to 2.5 cm) and starts light green before becoming brown and lignified/glabrous.

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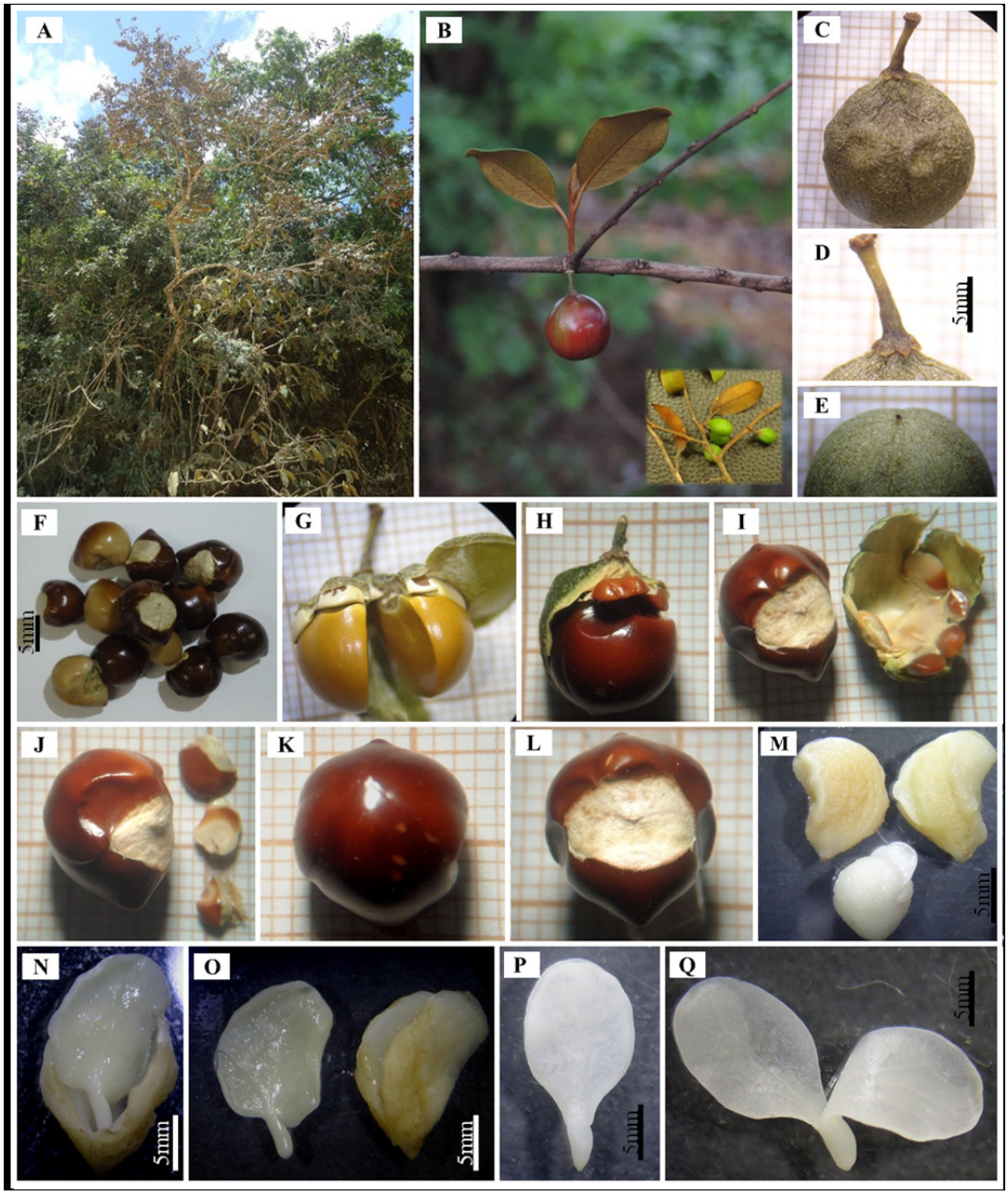

Figure 1. Images showing individuals of the species of *C. rufum*: A – habitat; B – fruit in maturation phase, with details showing fruits still immature; C, D and E: detail of dry fruit, base and apex, respectively; F – color and seed shape; G and H: number of seeds developed and viable per fruit; I and J: viable seed, more details showing undeveloped seeds; K and L: seed apex and base, displaying the hilum; M: endosperm covering the axis hypocotyl-root; N, O and P: endosperm and embryo detail; Q – detail showing the two foliaceous cotyledons.

Foliaceous cotyledons are 1.53 to 1.66 cm and photosynthetic and have a petiole that is greenish, opposite, glabrous and 0.26- to 0.31-cm in length. The epycotyl is 0.4 to 0.55 cm long and greenish, becoming light brown with indument pubescent-depressed and with white trichomes becoming ferruginous during its development (Figure 2). Internodes range from 4.64 to 8.91 mm and are greenish in color, with indument and texture similar to that of the epycotyl. The first leaves (16.61-27.10 x 9.14-9.84 mm) are simple, oblanceolate, cartaceous, alternating, and slightly spiraled, while the petiole (2.27 to 3.90 mm) is light green, not canaliculated, and pubescent-depressed, with ferruginous trichomes. Leaf blades are green, smooth on both sides, glossy and glabrescent, with rare ferruginous trichomes near the base and in the mid rib. In the bud and in the beginning of leaf development, trichomes are whitish, becoming ferruginous to golden; leaf bases are cuneate to slightly cuneate, with a cuspidade-acute apex, entire margin (rarely with ciliated margin, trichomes caduceous), and brochidodromous venation. In the beginning of the development of the first leaves (eophyll and metaphyll), only the primary and the secondary venation (arched near the margin) are evident. The following leaves present five to ten pairs of secondary veins, rare intersecondary and reticulate terciary veins, and a small amount of viscous white latex. A greenish apical bud, always formed by leaf sketches of different sizes, parallels a slightly curved, pubescent indument, with white to ferrugineous malpighiaceaous trichomes

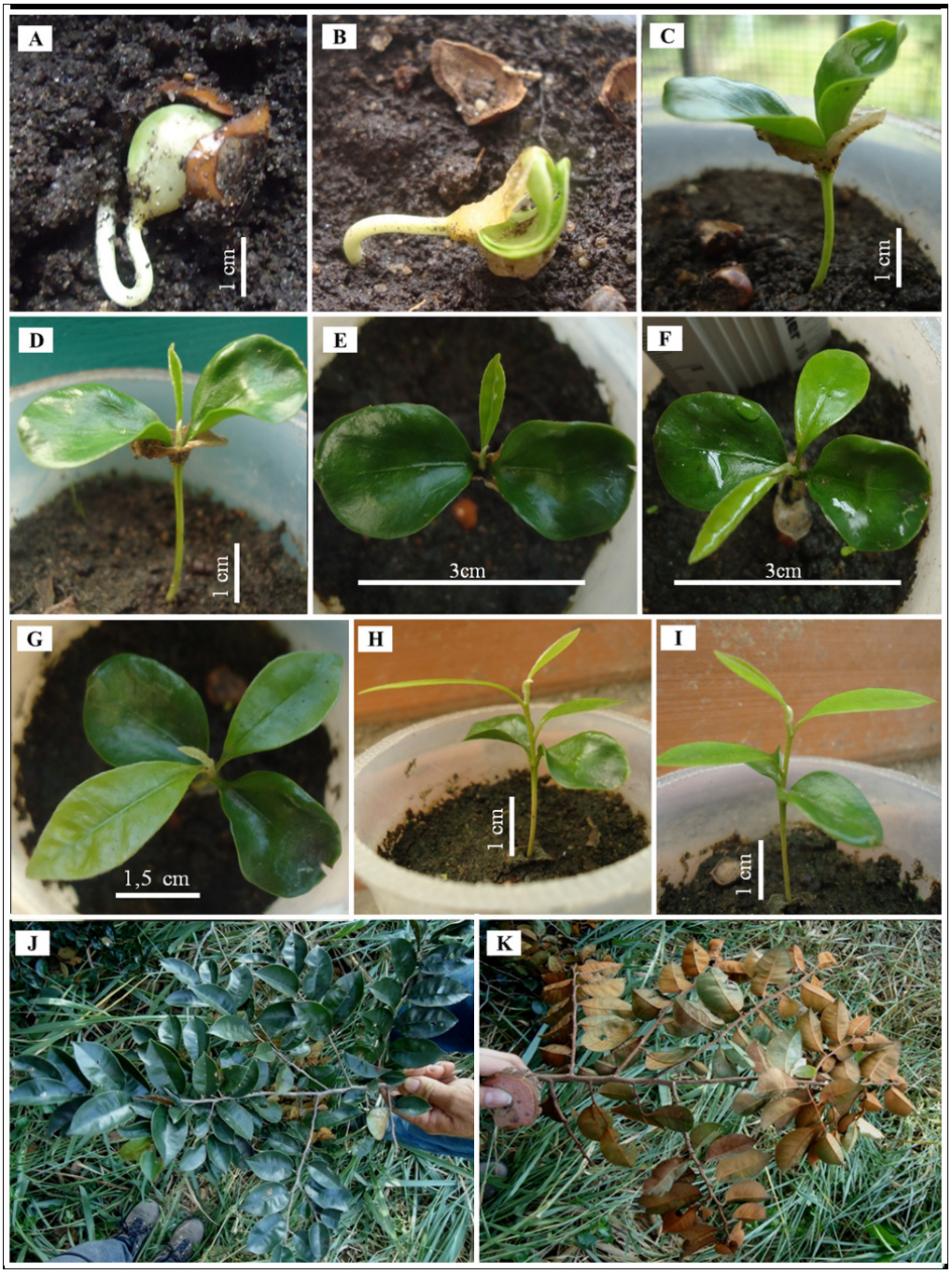

Figure 2. Germination and initial development of *C. rufum*: A and B: hypocotylar strap and early lifting of cotyledons; C – total lift of hypocotyl and expansion of foliaceous photosynthetic cotyledons; D and E: formation of first eophyll; F: formation of second eophyll; G, H and I: beginning of formation of the third eophyll until its complete expansion; J and K: branch of adult individual of *C. rufum,* showing the morphological differences (abaxial and adaxial side, respectively) from the seedling stage.

and largely asymmetric axes. The importance of seedling morphology studies is that many characteristics of young individuals are lost during their development, making the identification of those that germinate in forest areas difficult (especially for those developing research with soil seed bank and seedlings analysis) (Table 1, Figure 3). In the case of the species analyzed here, *Chrysophyllum rufum*, it was possible to observe some characteristics present in the seedling that can distinguish immature from adult individuals. The discussion about the importance of assessing the initial phase of plant organisms is not recent (Rizzini 1965, Ng, 1978, Vogel 1980, Candolle 1985, Miquel 1987). Studies related to the morphology of seedlings are useful in the restoration of anthropic areas or after disturbances and openings of forest clearings, as allowing the correct delimitation of taxa, even young individuals, contributes to support forestry strategies and, consequently, to accelerate the management of conservation actions (Almeida Jr. et al., 2010, Barreto & Ferreira 2010, Lima et al., 2010, Amorim et al., 2006, Guerra et al., 2006). In the case of *C. rufum*, the leaf variation, the amount and color of indumenta and embryo characteristics, and fruit and seed morphology all contribute to the identification of the species in its natural environment and aid its delimitation.

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Table 1. Primary differences observed in leaf morphology between seedling and adult individuals of *Chrysophyllum rufum* (Sapotaceae).

Characters	Seedling	Adult individual
Leaf shape	Oblanceolate	Oblong-elliptic (lanceolate)
Leaf consistency	Cartaceous	Coriaceous
Leaf apex	Cuneate to closely cuneate	Acute-acuminate
Leaf base	Cuneate to closely cuneate	Base obtuse-cuneate
Leaf margin	Entire or ciliated (trichomes caduceus)	Strongly revolute
Type of venation	Barely visible initially, then brochidodromous	Eucamptodromous-brochidodromous
Nº of pairs of secondary veins	5 to10	8 to 11
Indument of abaxial face	Glabrescent/ glabrous	Densely tomentose
Color of trichomes	Golden-whitish	Ferruginous to dark brown
Latex	Small amounts	More abundant

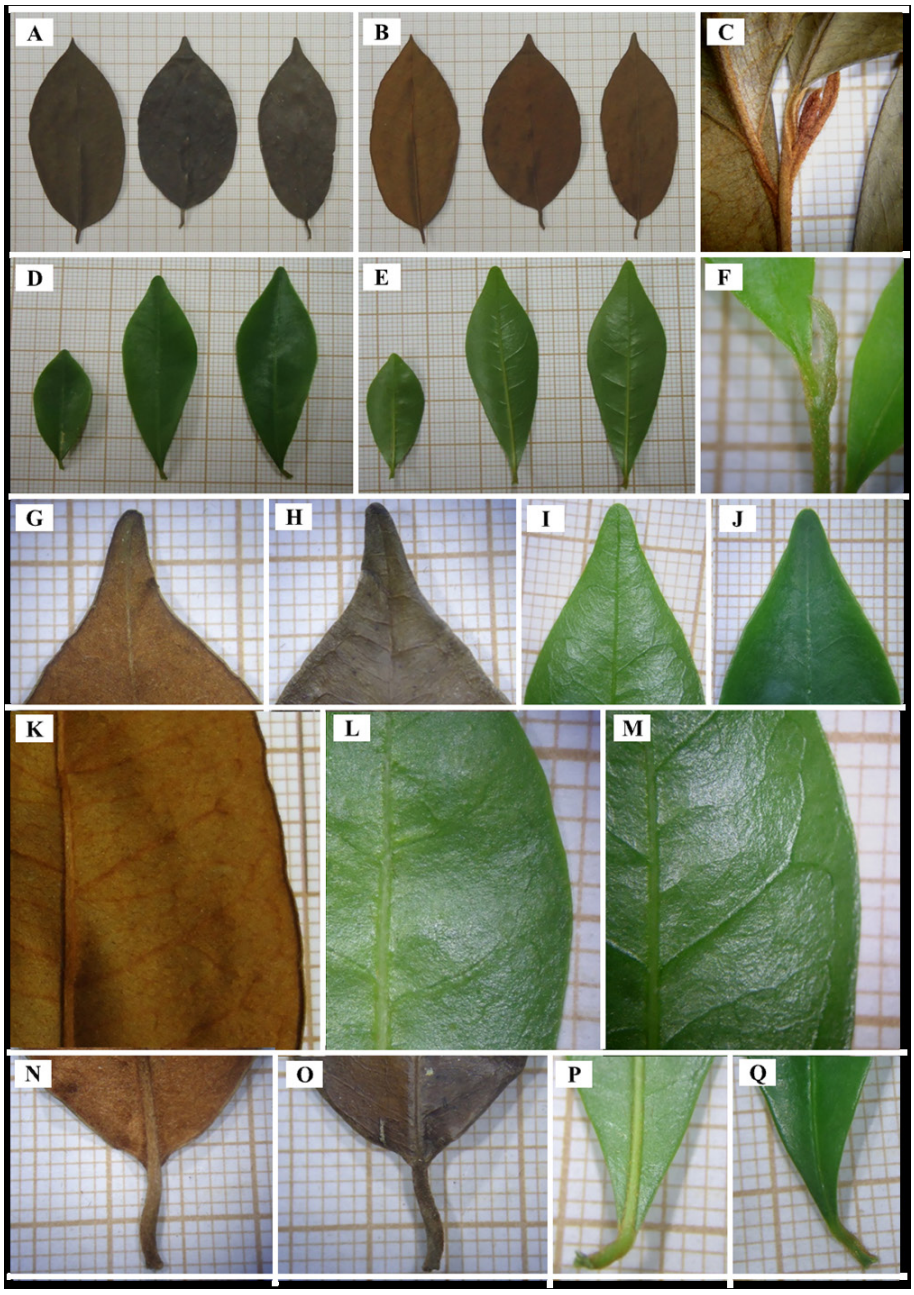

Figure 3. Comparison of leaf morphology between adult individual and seedling: A, B and C: leaf shape (abaxial and adaxial side, respectively) and bud of adult individual; D, E and F: leaf form (abaxial and adaxial side, respectively) and bud of seedling; G, H, I and J: leaf apex of adult individual and seedling; K: leaf venation of adult individual; L and M: leaf venation during seedling development; N, O, P, Q: leaf base of adult individual and seedling.

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Author Contributions

Liliane Ferreira Lima: collection and species identification, analysis and monitoring of the experiment in the greenhouse and laboratory, orientation methodology, and theoretical contributions; substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision augmenting intellectual content.

Renata Gabriela Vila Nova de Lima: support during field collection, practical execution of the experiment in the greenhouse and laboratory, and assistance with personnel and scientific development; substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation.

Angélica Cândida Ferreira: support during field collection, practical execution of the experiment in the greenhouse and laboratory, and assistance with personnel and scientific development; substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation.

Eduardo Bezerra de Almeida Junior: support for orientation methodology and scientific theoretical contributions; contribution to manuscript preparation; contribution to critical revision augmenting intellectual content.

Carmen Sílvia Zickel: support for orientation methodology and scientific theoretical contributions; contribution to manuscript preparation; contribution to critical revision augmenting intellectual content.

Conflicts of interest

The authors declare that there is no conflict of interest related to the publication of the data in this article.

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