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Morphological characterization of fruits and seeds and post-seminal development of *Myrcia retorta* Cambess

Caracterização morfológica de frutos e sementes e do desenvolvimento pós-seminal de *Myrcia retorta* Cambess

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Highlights

Micro-CT is a valuable tool for evaluating *Myrcia retorta* seeds.
The number of seeds per fruit influences seedling vigor.
First scientific report describing the fruits and seeds of *Myrcia retorta.*

Abstract

Myrcia retorta (Myrtaceae) is a small native Brazilian tree, commonly known as guamirim, found mainly in the Atlantic Forest and Cerrado biomes. It plays a crucial role in these ecosystems by providing food and shelter for various animal species. Additionally, it is valued in landscaping and for its medicinal properties. To expand our knowledge of this species, we aimed to characterize the morphology of its fruits and seeds and assess seed germination and vigor. Understanding these characteristics is essential for species identification and provides a foundation for studies in plant organography and systematics. For this purpose, seeds were classified into two batches: (1) spherical seeds from monospermic fruits and (2) from dyspermic fruits. One hundred fruits were sampled, and the following characteristics were evaluated: dimensions (length, width, and thickness), texture, color, dehiscence, pericarp consistency, and number of seeds per fruit. Both batches were subjected to X-ray micro-computed tomography to assess cotyledon type, hypocotyl-radicle axis, embryo type, endosperm presence, and seed dimensions. Germination tests were conducted to determine germination type, germination rate, and germination speed index (GSI). Our results indicate that *M. retorta* produces bacaceous fruits that are monospermic, dyspermic, or trispermic, spherical in shape, and vinaceous in color. Germination is

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epigeal and phanerocotylar. Germination in batch 1 was 51% with a GSI of 2.05, whereas in batch 2, germination was 52% with a GSI of 1.75. The use of monospermic or dyspermic fruits is recommended for seedling production. Additionally, X-ray micro-computed tomography proved to be an effective, non-destructive technique for analyzing the internal and external morphology of *M. retorta* seeds.
Key words: Brazilian flora. Germination. Micro-CT. Myrtaceae. Native trees.

Resumo

Myrcia retorta (Myrtaceae) é uma pequena árvore nativa do Brasil, comumente conhecida como guamirim, encontrada principalmente nos biomas Mata Atlântica e Cerrado. Desempenha um papel fundamental nesses ecossistemas, fornecendo alimento e abrigo para diversas espécies animais. Além disso, é valorizada no paisagismo e por suas propriedades medicinais. Para ampliar o conhecimento sobre esta espécie, objetivamos caraterizar a morfologia de seus frutos e sementes e avaliar a germinação e o vigor das sementes. A compreensão dessas caraterísticas é essencial para a identificação das espécies e fornece bases para estudos em organografia e sistemática vegetal. Para isso, as sementes foram classificadas em dois lotes: (1) sementes esféricas de frutos monospérmicos e (2) de frutos dispérmicos. Foram amostrados 100 frutos e avaliadas as seguintes caraterísticas: dimensões (comprimento, largura e espessura), textura, cor, deiscência, consistência do pericarpo e número de sementes por fruto. Ambos os lotes foram submetidos à microtomografia computadorizada de raios X para avaliação do tipo de cotilédone, eixo hipocótilo-radícula, tipo de embrião, presença de endosperma e dimensões da semente. Foram efectuados testes de germinação para determinar o tipo de germinação, a taxa de germinação e o índice de velocidade de germinação (GSI). Os nossos resultados indicam que *M. retorta* produz frutos bacáceos monospérmicos, dispérmicos ou trispérmicos, de forma esférica e de cor vinácea. A germinação é epígea e fanerocotílica. A germinação no lote 1 foi de 51% com um GSI de 2,05, enquanto no lote 2, a germinação foi de 52% com um GSI de 1,75. Recomenda-se o uso de frutos monospérmicos ou dispérmicos para a produção de mudas. Além disso, a tomografia microcomputada de raios X provou ser uma técnica eficaz e não destrutiva para analisar a morfologia interna e externa das sementes de *M. retorta.*
Palavras-chave: Árvores nativas. Flora brasileira. Germinação. Micro-CT. Myrtaceae.

Introduction

The Myrtaceae family (Order Myrtales) is widely represented in Brazilian flora, occurring across various biomes, and comprising 23 genera and 1,026 species. It is one of the most significant families of Angiosperms in Brazil, recognized as the richest or among the richest in terms of tree species diversity (Wagner & Fiaschi, 2020; Silveira et al., 2021). This family encompasses a vast diversity of plant species, including trees, shrubs, and herbs, making it one of the largest groups in Brazilian botany.

From an economic perspective, Myrtaceae plays a crucial role in Brazil. It is a major supplier of raw materials for the pulp and paper industry, particularly through the genus Eucalyptus, which is extensively cultivated in commercial plantations across the country. Additionally, fruit-bearing species such as *Psidium guajava, Plinia cauliflora,* and *Eugenia uniflora* are commercially exploited and hold

economic importance in both domestic and export markets.

Among the best-represented genera within Myrtaceae is *Myrcia,* the fourth-largest genus in this family, comprising approximately 770 species distributed from Central America and the Caribbean to southern South America. In Brazil, Myrcia includes 397 species, of which 309 are endemic, occurring primarily in the Atlantic Forest and Amazon domains, with lesser representation in the Caatinga and Cerrado biomes (Lourenço et al., 2020; Lannoy et al., 2021). Many species within this genus exhibit high medicinal potential, with studies reporting antimicrobial, anti-inflammatory, antioxidant, and anti-hemorrhagic activities (Moraes et al., 2022; Ribeiro et al., 2022; Sá et al., 2017).

However, it is essential to emphasize that the significance of the Myrtaceae family extends beyond its economic value. Many of its species play critical roles in natural ecosystems by providing habitat and food for various organisms while contributing to climate regulation and biodiversity conservation (Freitas et al., 2018).

A notable example in this ecological context is *Myrcia retorta* Cambess., an endemic Brazilian species described as a shrub or small tree reaching up to 6 meters in height. Its leaves are simple, opposite, coriaceous, and generally elliptical. The species is easily identified by its young leaves, which exhibit a brownish indumentum on the abaxial surface with whitish punctuations that gradually turn blackish and detach easily upon touch. Its flowers are small, white, and arranged in axillary inflorescences (Rosa & Romero, 2012).

M. retorta occurs in various vegetation types, including dense Ombrophilous forests, semideciduous seasonal forests, and cerrado. Its geographic distribution is concentrated in Brazil, particularly in the Atlantic Forest and Cerrado biomes, with a higher prevalence in the states of Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, and Minas Gerais. The species has a distribution center in Santa Catarina and Paraná (Carvalho, 2008).

Beyond its ecological importance, *M. retorta* possesses medicinal potential and is widely used in traditional medicine for various applications. Its leaves, bark, and fruits are utilized in medicinal preparations for treating gastrointestinal disorders, inflammations, and other conditions. Like other Myrtaceae species, *M. retorta* plays a crucial role in ecosystem dynamics. Its flowers are frequently visited by bees and other pollinating insects, while its fruits serve as a food source for birds and mammals, facilitating seed dispersal. This trait makes the species valuable for ecological restoration and landscaping purposes (Cascaes et al., 2015).

Despite its significance, *M. retorta,* like many other native species in Brazil, faces threats from habitat loss and environmental degradation. Conserving its natural habitat is essential to ensuring the survival of this species and the biodiversity associated with it. In this context, the limited knowledge regarding the morphology of its fruits and seeds represents a significant gap. Expanding this understanding is crucial not only for elucidating the species' life cycle and growth patterns but also for developing effective management and seedling production techniques (Ferreira et al., 2013).

Morphological studies, combined with technological advancements such as X-ray micro-computed tomography (micro-CT), have proven highly promising. This non-destructive technique employs high-resolution imaging to generate three-dimensional reconstructions of plant structures from multiple angles, allowing detailed visualization of internal seed morphology. The application of image analysis to seeds has evolved into a valuable technological tool for precise, non-destructive evaluations, enabling the observation of internal structures and subsequent performance assessments (Guariz et al., 2022).

Given the need for studies addressing the morphological description and germination processes of ecologically and economically valuable native species, this study aimed to describe the morphology of the fruits, seeds, and seedlings of *M. retorta* using X-ray micro-computed tomography. Additionally, it sought to assess the physiological quality of seeds through germination and vigor analyses.

Materials and Methods

The study was conducted at the Seed Analysis Laboratory of the Agricultural Sciences Center, State University of Londrina, in Londrina, Paraná, Brazil. Fruits were manually collected using a ladder and pruning shears at Morro da Pedra Branca, Serra do Cadeado, Ortigueira, Paraná (23°58'02"S; 51°04'33" W) in May 2022. At the time of collection, the fruits exhibited visible signs of maturity, identified by pulp texture and the vinaceous color of the epicarp (Figure 1A).

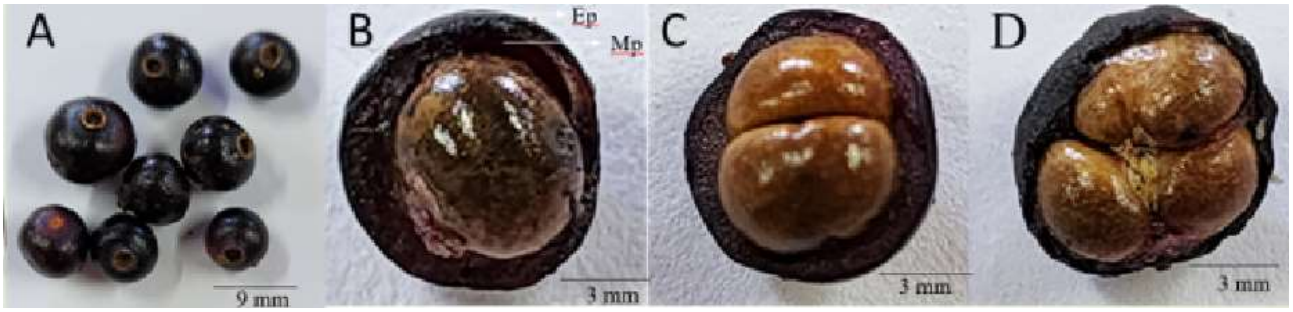

Figure 1. (A) Whole, ripe fruits of *Myrcia retorta* Cambess. (B) Monospermic fruit with distinct epicarp and mesocarp. (C) Dispermous fruit. (D) Trispermous fruit. (*ep:* epicarp; *mp:* mesocarp).

The seeds were depulped by rubbing the fruits against plastic sieves under running water to ensure complete pericarp removal. They were then stored in airtight plastic bags at room temperature, following the method described by Pedro (2024). After processing, the seeds were categorized into two batches based on external morphological characteristics: Batch I – spherical seeds from fruits containing a single seed, and Batch II – seeds with a flattened face, forming a semicircle, from fruits containing two seeds (Figures 1B, C, and D). Fruits containing one or more seeds were observed in all analyzed parent plants.

A random sample of 100 fruits and 100 seeds from the collected specimens was used. For the morphological description, only intact, mature, non-deformed, and healthy fruits were selected. The classification of fruit type, dimensions, texture, color at maturity, pericarp consistency, seed position within the fruit, and seed number per fruit was performed using a stereoscopic microscope.

Morphometric measurements of fruits and seeds (length, width, and thickness) were taken using a digital caliper with an accuracy of 0.001 mm. Seed morphology was described under a stereoscopic microscope, considering both internal characteristics (color, cotyledon presence and type, hypocotyl-root axis, embryo type, endosperm presence, and seed coat composition) and external characteristics (consistency, color, presence of spots, seed coat texture, and shape).

Internal seed measurements were obtained using X-ray micro-computed tomography (micro-CT) with a SkyScan-Bruker microtomograph (model 1173), operating at 45 kV, 226 µA current, 4 µm image resolution, and 0.25° angular steps over 180°. To enhance imaging clarity, the seeds were dehydrated using an alcohol gradient, with exposure for 20 minutes at each concentration: 0, 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, and 100%.

Germination test

After seed extraction, all seeds were disinfected by immersion in a 1% sodium hypochlorite (NaClO) solution for 5 minutes. The experiment followed a completely randomized design with two treatments: Treatment 1 – spherical seeds from monospermic fruits, and Treatment 2 – seeds with a flattened face from dispermous fruits. Each treatment included four replicates of 50 seeds.

Seed germination was conducted in a Mangelsdorf germinator at a constant temperature of 25°C. Seeds were placed on two sheets of blotting paper, with distilled water added in an amount equal to 2.5 times the paper's weight to maintain humidity (Ministério da Agricultura, Pecuária e Abastecimento, [MAPA, 2009]) Measurements were recorded daily until germination stabilized.

Seeds were considered germinated when radicle protrusion exceeded 2 mm. At the end of the experiment, daily germination data were used to calculate the following parameters, as proposed by T. C. Ferreira et al. (2022): Germination Percentage (G%) or Germinability, and Germination Speed Index (GSI).

Results

Fruit morphology

Myrcia retorta produces baccate, indehiscent, and fleshy fruits. The fruits are globose, with a smooth, shiny, and glabrous epicarp that is thin, light green when immature, and dark purple when mature (Figure 1A). The mesocarp is fleshy, light purple, and semi-transparent, while the endocarp is not visible.

Fruits can be monospermic (the most common form) (Figure 1B), dispermous (Figure 1C), or trispermous (Figure 1D), with seeds positioned in the medial region. The fruits had an average length of 7.00 mm (ranging from 5.50 to 9.19 mm), an average width of 7.08 mm (ranging from 4.95 to 9.24 mm), and an average thickness of 7.04 mm (ranging from 5.19 to 9.64 mm). The dimensions, standard deviation, median, and number of seeds per fruit are presented in Table 1.

Table 1
Size and seed number per Myrcia retorta Cambess fruit

Dimension (mm)	Average	Standard deviation	Median	Minimum	Maximum
Length	7.00	0.86	6.97	5.5	9.19
Width	7.08	0.96	7.00	4.95	9.24
Thickness	7.03	0.97	7.04	5.14	9.64
Number of seeds	1.43	0.68	1	1	1

Seed morphology

The seed coat is flexible and easily removable, featuring both a hilum and a micropyle, the latter characterized as an orifice with a lateral indentation. The coat is smooth, shiny, and green with pinkish-brown or yellowish spots. It is laterally compressed, with a rounded base and apex. The hilar scar is white with a pink outline. Micro-CT imaging revealed the presence of two seed coat layers: the testa, the outermost layer, and the tegmen, the innermost layer (Figure 2A).

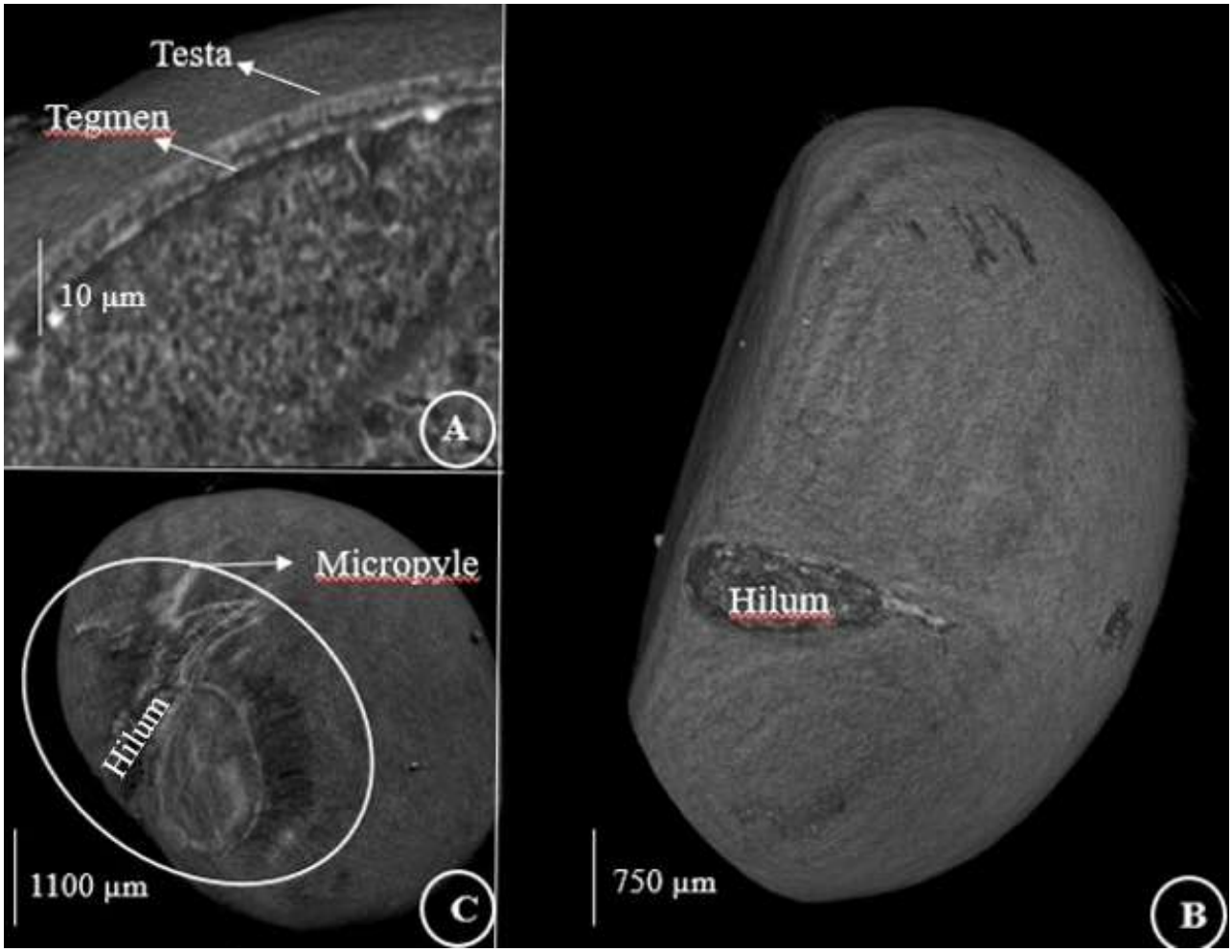

Figure 2. X-ray micro-computed tomography of *Myrcia retorta* Cambess. (A) Seed coat region showing its two layers. (B) Seed from batch 2 with a flattened side and visible hilum. (C) Spherical seed from Batch 1 showing the micropyle and hilum.

The external seed morphology varies depending on the number of seeds per fruit. Monospermic seeds are obovate or spherical, while seeds from dispermous or trispermous fruits exhibit shape variations, being either laterally compressed or flat-convex (Figures 2B and 2C).

The embryo is mycetoid, light green, and invaginated, with well-developed and strongly folded cotyledons. The hypocotyl-root axis is positioned on the dorsal side of the cotyledons (Figure 3). Its position varies slightly between batches: in Batch 1, it is centered in the dorsal region, while in Batch 2, due to compression during seed formation, it is slightly displaced toward the flattened face (Figure 2B). A small amount of mucilaginous endosperm was observed, visible only after germination. Additionally, spherical structures were noted throughout the seed interior, particularly within the cotyledons (Figure 3).

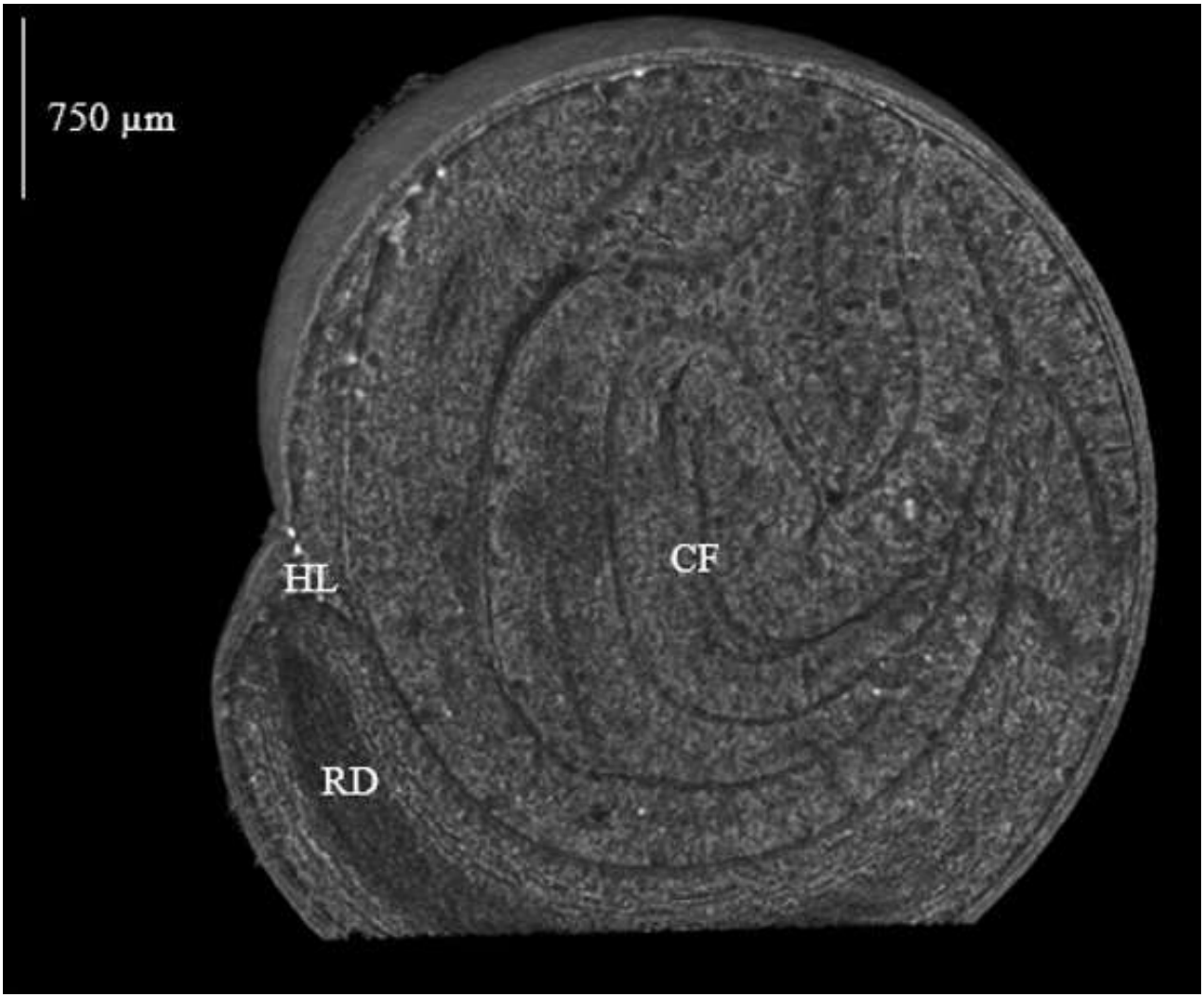

Figure 3. Micro-CT image of a longitudinally sectioned *M. retorta* seed, revealing foliaceous cotyledons.

The seed measurements for each batch were as follows: Batch 1: Average length of 4.63 mm (ranging from 3.89 to 5.25 mm), average width of 4.17 mm (ranging from 3.20 to 5.17 mm), and average thickness of 4.00 mm; Batch 2: Average length of 5.00 mm (ranging from 3.79 to 6.25 mm), average width of 3.49 mm (ranging from 1.96 to 5.18 mm), and average thickness of 4.14 mm (ranging from 3.08 to 5.25 mm).

The mean, minimum, and maximum dimensions, median, and standard deviation are presented in Table 2. Batch 2 exhibited greater standard deviations across all three parameters, reflecting a wider range of minimum and maximum dimensions compared to Batch 1 (Table 2).

Table 2
Dimensions of two Myrcia retorta seed batches

Dimension (mm)		Average	Standard deviation	Median	Minimum	Maximum
Batch 1	Length	4.63	0.42	4.72	3.89	5.25
	Width	4.13	0.49	4.11	3.2	5.17
	Thickness	4.05	0.51	4.14	3.04	5.06
Batch 2	Length	5.00	0.58	5.13	3.79	6.25
	Width	3.49	0.84	3.44	1.96	5.18
	Thickness	4.14	0.67	4.19	3.08	5.25

Germination test

Germination of *Myrcia retorta* seeds is phanerocotylar epigeal, meaning the cotyledons fully emerge above the soil surface during germination. The term "phanero" denotes something visible or evident, indicating that the cotyledons remain exposed, while "epigeal" refers to the initial growth of the seedling occurring above the soil surface (MAPA, 2009).

M. retorta seeds were considered germinated two days after sowing (DAS). During seedling development, three distinct regions could be identified: the root region, the collar, characterized by abundant root hairs, and the hypocotyl, which began elongating nine DAS. As elongation progressed, the seed coat ruptured, and the cotyledons, still heavily wrinkled, emerged around 15 DAS. Approximately four days after cotyledon release, they fully expanded (Figure 4).

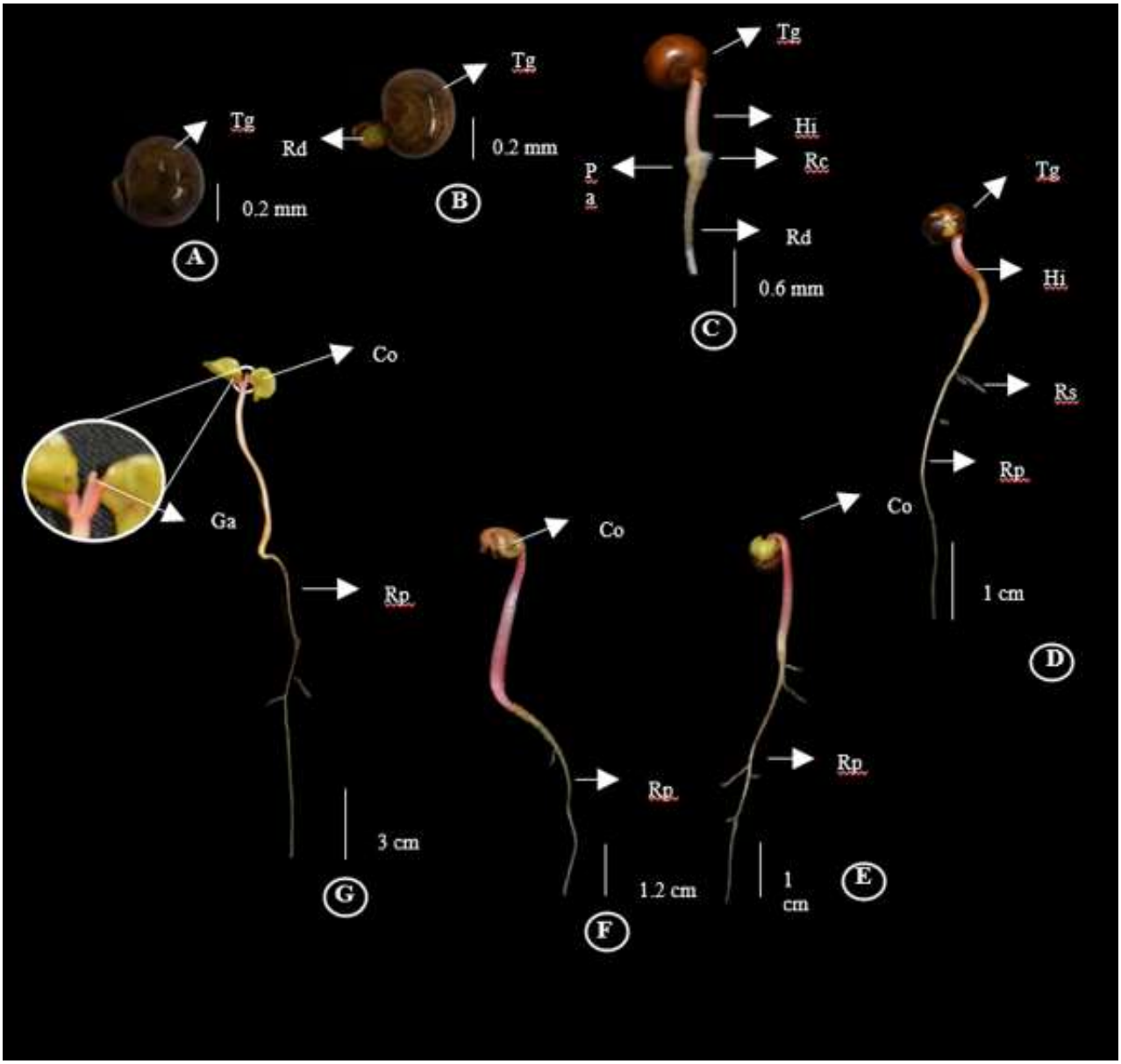

Figure 4. Morphology and germination process of *Myrcia retorta* Cambess. (A) Seed with ruptured seed coat. (B) Seed with ruptured seed coat and radicle protrusion. (C) Hypocotyl with a well-defined collar region and absorbent hairs. (D) Seedling with secondary root formation. (E) Seedlings with cotyledons nearly free from the seed coat. (F) Seedling with wrinkled cotyledons shortly after emerging from the seed coat. (G) Seedling with fully expanded cotyledons and an emerging apical bud. (Rd: radicle; Pa: absorbent hairs; Hi: hypocotyl; Rp: primary root; Co: cotyledons; Ga: apical bud).

Following complete cotyledon expansion, the apical bud developed, and the first pair of eophylls, resembling adult leaves (metaphylls), emerged. The germination percentage was 51% for Batch 1 and 52% for Batch 2, indicating that seed morphology did not influence this parameter. The Germination Speed Index (GSI) was 2.05 for Batch 1 and 1.75 for Batch 2.

Discussion

M. retorta produces fleshy, baccate fruits that are often edible and highly attractive to animals, facilitating seed dispersal (zoochory) (Stefanello et al., 2010). The fruits are spherical. According to Morais and Lombardi (2006), they are globose, pilose, and crowned by remnants of the calyx lobes and hypanthium, with zoochorous dispersal.

Santos and Sano (2012) describe mature fruits as red to black, globose, glabrescent to glabrous, glandular-warty, with a spreading or reflexed calyx, typically containing a single seed with a crustaceous testa. Bünger et al. (2012) report that fruits are approximately 4.0 mm in diameter, globose, and sparsely pubescent.

The fruits of *M. retorta* differ from those of other *Myrcia* species, particularly in coloration and pulp presence. Despite belonging to the same genus, these morphological differences facilitate easy species identification in the field. Emer et al. (2018) highlight that fruit coloration in Myrtaceae species serves as a reliable indicator of the optimal collection point for seed extraction a critical factor in this study, where fruit collection was determined by dark vinaceous coloration, indicative of viable seeds.

A comparative survey of fruit characteristics among *Myrcia* species in Brazil can be conducted. As observed, *M. retorta* exhibits light green fruits when immature and dark purple fruits when mature. For other species, Silva et al. (2020) reported the following: *Myrcia aethusa* produces yellow fruits; *Myrcia anacardiifolia* has yellow to bright red fruits with pulp; and *Myrcia antonia* bears hairy fruits that mature to a brownish or yellowish color.

Further comparisons reveal morphological fruit traits, as well as variations in seed germination, establishment, and ecological adaptations. Studies suggest that the resilience and potential for ecological restoration of *M. retorta* are closely tied to these characteristics.

M. retorta is currently classified as "Least Concern" (LC) by the International Union for Conservation of Nature (IUCN) (Negrão et al., 2020). However, ongoing deforestation, fires, and mining activities pose potential threats, particularly in the Atlantic Forest. Further research is needed to assess its current population size and distribution, as well as nursery production efforts to support conservation initiatives.

Conclusions

The rapid germination of *Myrcia retorta* seeds highlights seed propagation as an effective strategy for preserving the genetic diversity of native forest species. X-ray micro-computed tomography proved to be a reliable tool for analyzing seed morphology. For silvicultural purposes, seeds from monospermic fruits are recommended for higher seedling quality.

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