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# Morphologic and Morphometric Differences between *Cenostigma pyramidale* and *Cenostigma microphyllum* Seeds

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## Abstract

The characterization of *Cenostigma pyramidale* and *Cenostigma microphyllum* seeds for image measurements is important for improving knowledge of Caatinga areas. Accordingly, this research aimed to compare the seeds of *C. pyramidale* and *C. microphyllum* in terms of morphological and morphometric traits, using digital image analysis as a method for measuring and classifying the seeds. *C. pyramidale* and *C. microphyllum* seeds were analysed for morphological and morphometric characters. The results obtained were analysed for descriptive statistics and non-parametric methods. *C. pyramidale* and *C. microphyllum* exhibit similar morphological characteristics, but show significant differences in seed shape and size variability. Morphometric indices, combined with multivariate statistical analysis, allowed for a clear distinction between them. The findings support the use of morphometric parameters as consistent tools for species discrimination, with direct applications in ecological restoration, seed technology, and biodiversity conservation in the Caatinga areas.

**Keywords:** Brazilian dry season forest, Caatinga, diversity, forestry

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## Diferencias morfológicas y morfométricas entre las semillas de *Cenostigma pyramidale* y de *Cenostigma microphyllum*

### Resumen

La caracterización entre semillas de *Cenostigma pyramidale* y de *Cenostigma microphyllum* para mediciones de imágenes es importante para mejorar el conocimiento sobre las áreas de Caatinga. En consecuencia, esta investigación tuvo como objetivo comparar las semillas de *C. pyramidale* y *C. microphyllum* en términos de rasgos morfológicos y morfométricos, utilizando el análisis de imágenes digitales como método para medir y clasificar las semillas. Se analizaron las semillas de *Cenostigma pyramidale* y *Cenostigma microphyllum* para caracteres morfológicos y morfométricos. Los resultados obtenidos se analizaron para estadísticas descriptivas y métodos no paramétricos. *Cenostigma pyramidale* y *C. microphyllum* exhiben características morfológicas similares, pero muestran diferencias significativas en la variabilidad de la forma y el tamaño de las semillas. Los índices morfométricos, combinados con el análisis estadístico multivariado, permitieron una clara distinción. Los hallazgos respaldan el uso de parámetros morfométricos como herramientas consistentes para la discriminación de especies, con aplicaciones directas en la restauración ecológica, la tecnología de semillas y la conservación de la biodiversidad en las áreas de Caatinga.

**Palabras clave:** bosque seco brasileño, Caatinga, diversidad, silvicultura

## Diferenças morfológicas e morfométricas entre sementes de *Cenostigma pyramidale* e *Cenostigma microphyllum*

### Resumo

A caracterização entre sementes de *Cenostigma pyramidale* e *Cenostigma microphyllum* para medições de imagens são importantes para melhorar o conhecimento sobre as áreas de Caatinga. Assim, esta pesquisa teve como objetivo comparar as sementes de *C. pyramidale* e *C. microphyllum* em termos de características morfológicas e morfométricas, usando análise de imagem digital como método para medir e classificar as sementes. Sementes de *Cenostigma pyramidale* e *C. microphyllum* foram analisadas para caracteres morfológicos e morfométricos. Os resultados obtidos foram analisados por estatísticas descritivas e métodos não paramétricos. *C. pyramidale* e *C. microphyllum* exibem características morfológicas semelhantes, mas mostram diferenças significativas na forma da semente e na variabilidade do tamanho. Índices morfométricos, combinados com análise estatística multivariada, permitiram uma distinção clara entre eles. Os resultados apoiam o uso de parâmetros morfométricos como ferramentas consistentes para discriminação de espécies, com aplicações diretas em restauração ecológica, tecnologia de sementes e conservação da biodiversidade nas áreas de Caatinga.

**Palavras-chave:** floresta seca brasileira, Caatinga, diversidade, silvicultura

## 1. Introduction

The Brazilian Seasonally Dry Tropical Forests (Caatinga areas) are a vegetation formation of great relevance to Brazil due to their economic, social, and ecological importance. These forests are remarkable for their species richness, high levels of endemism, and diverse strategies for survival and dispersion of seeds (Albuquerque et al., 2024). However, due to human-driven degradation and climate change, this region is experiencing biodiversity loss, a decline in ecological functionality, and increasing desertification (Arraes et al., 2024).

Several endemic species are found in the Caatinga areas, such as Fabaceae species *Cenostigma pyramidale* (Tul.) Gagnon & G.P. Lewis (Silva et al., 2024) and *Cenostigma microphyllum* (Mart. ex G. Don) Gagnon & G.P. Lewis (Nóbrega et al., 2024). These species should be investigated for a variety of purposes, particularly their genetic variability, which remains poorly understood in some cases (Aecyo et al., 2025; Arraes et al., 2024; Castro et al., 2024; Patrício & Trovão, 2020).

*Cenostigma pyramidale* is a key tree species due to its ecological and economic relevance as a pioneer species (Silva et al., 2024), its resistance to harsh environmental conditions (Vilela et al., 2021), good tolerance to salinity (Nóbrega et al., 2024), and resilience during dry periods (Gomes et al., 2019). It is widespread across many areas of the Caatinga lands (Patrício & Trovão, 2020) and is important for research in sectors such as forestry, forage production, and the restoration of degraded areas (Aecyo et al., 2025; Arraes et al., 2024; Castro et al., 2024).

*Cenostigma microphyllum* also occurs in several parts of the Caatinga areas (Gomes et al., 2019). According to H. Oliveira et al. (2023), this species is valuable for reforestation but is sensitive to extreme environmental conditions (H. Oliveira et al., 2023; W. Oliveira et al., 2023). Despite its broad distribution, it remains poorly studied, particularly with regard to its ecological interactions (W. Oliveira et al., 2023).

The diversity of *Cenostigma pyramidale* and *Cenostigma microphyllum* specimens is debated in the literature (Aecyo et al., 2025; Castro et al., 2024). Therefore, a more refined analysis of these two species may play a significant role in advancing the understanding of Caatinga biodiversity (Arraes et al., 2024; Patrício & Trovão, 2020).

Research aimed at better understanding the biodiversity of Caatinga diaspores is increasingly important in light of the environmental degradation and desertification this area is undergoing (Arraes et al., 2024; Patrício & Trovão, 2020). A key approach to studying species diversity via seeds is image-based morphometric analysis, which can be a decisive tool for advancing knowledge about forest ecosystems. This method is grounded in the premise that the diversity of diaspores reflects the characteristics of the forests that produce them (Felix, Demartelaere, et al., 2023; Felix, Kratz, et al., 2023; Felix et al., 2018, 2024).

Accordingly, this study aimed to compare the seeds of *C. pyramidale* and *C. microphyllum* in terms of morphological and morphometric traits, using digital image analysis as a method for measuring and classifying the seeds.

## 2. Materials and Methods

The research was conducted at the Campina Grande Federal University, located in Campina Grande city, Paraiba state, in March of 2025.

The *C. pyramidale* and *C. microphyllum* seeds were provided to the Center for Ecology and Environmental Monitoring - NEMA/UNIVASF, the Project for the Integration of the São Francisco River with the Hydrographic Basins of the Northern Northeast - PISF, and the Ministry of Integration and Regional Development - MIDR. *C. pyramidale* (Juazeiro municipality, Bahia state −9,605072135 and −40,45131409) and *C. microphyllum* (Juazeiro municipality, Bahia state −8,123306568 and −37,43604565) were collected in 2022 and stored in plastic bags in a temperature chamber at 15 °C.

The morphological description of the analyzed species was based on the observation of visible seed traits, such as general shape, color tone, surface texture, seed coat firmness, as well as the presence and configuration of the pleurogram, hilum, and micropyle, among other structures perceptible to the naked eye. The approach followed procedures inspired by Leitão et al. (2024), with terminology adopted according to the guidelines of Brazil (Castro et al., 2024; Patrício & Trovão, 2020; Secretaria de Defesa Agropecuária, 2013). For morphometric studies, seed imaging methods were employed, based on the protocols outlined by Loddo et al. (2023) and Felix et al. (2024). Images were captured using a Redmi Note 13 smartphone (Xiaomi Corporation, China), equipped with a 108 MP main camera (Samsung ISOCELL HM6 sensor), capable of

producing photos at a resolution of up to 12,000 × 9,000 pixels and recording 4K videos at 30 frames per second. The device was placed at a fixed distance of 50 cm from the samples, which were arranged on a navy-blue background with a millimeter-scale ruler for calibration and measurement purposes.

The acquired images were saved in JPEG format and processed using ImageJ® software (version 1.53; National Institutes of Health, USA). Morphometric measurements included metric variables: Area (AREA, mm²), Perimeter (PER, mm), Height (HEIG, mm), and Width (WIDT, mm) –as well as shape indices such as Circularity (CIR, 0.0-1.0), Roundness (ROUN, 0.0-1.0), and Solidity (SOL, 0.0-1.0) (Felix, Demartelaere, et al., 2023; Felix, Kratz, et al., 2023; Felix et al., 2018, 2024).

To confirm the existence of significant differences between the two species, a Multivariate Analysis of Variance (MANOVA) was performed. Following this, a Linear Discriminant Analysis (LDA) was used to identify which morphometric variables (Area, Perimeter, and Circularity) contributed most to the discrimination between *C. pyramidale* and *C. microphyllum*. All analyses were conducted using Past program, with a significance level of $p < 0.05$.

The resulting numerical data were subjected to descriptive statistical analysis (Mean, Standard Error, Variance, Standard Deviation, 25th Percentile, 75th Percentile, Skewness, Kurtosis, Coefficient of Variation) and Pearson Correlation tests, following the framework outlined by Divi et al. (2024). The box-plot graphics and analysis were made according to Abt et al. (2025).

The Simpson's Dominance and Diversity Indexes (1 - D) and Shannon Diversity Index were determined according to Ma (2025). In addition, we used the Principal Component Analysis (PCA) to understand the relationships between variables within each species, in accordance with Loddo et al. (2023).

All statistical procedures were performed using PAST 4.03 software (Paleontological Statistics), which has been validated for population-level studies involving seed measurement variables (Bezabih et al., 2023).

## 3. Results

The morphological characteristics of *Cenostigma pyramidale* and *Cenostigma microphyllum* seeds are described in Table 1 and Figure 1. The species are similar between them in terms of form and morphological characters, but differ in relation to color.

The Multivariate Analysis of Variance (MANOVA) confirmed significant differences between the seeds of the two species (Wilks' Lambda = 0.152; F = 145.4; $p < 0.001$). Furthermore, the Linear Discriminant Analysis (LDA) showed that Area and Circularity were the variables that contributed most to the separation of the groups, correctly classifying 98% of the seeds.

**Table 1.** Species-level characterization of *Cenostigma pyramidale* and *Cenostigma microphyllum* seeds

| Species | Brazilian Popular Name | Shape | Hilum Color | Micropyle Color | Hilum Shape | Surface | Seed Coat Texture | Tegument Color |
|---|---|---|---|---|---|---|---|---|
| *Cenostigma pyramidale* | Catingueira | Obovoid | Grey | Brown | Linear | Smooth | Hard | Dark brown |
| *Cenostigma microphyllum* | Catingueira-miuda | Obovoid | Grey | Brown | Linear | Smooth | Hard | Light greenish brown |

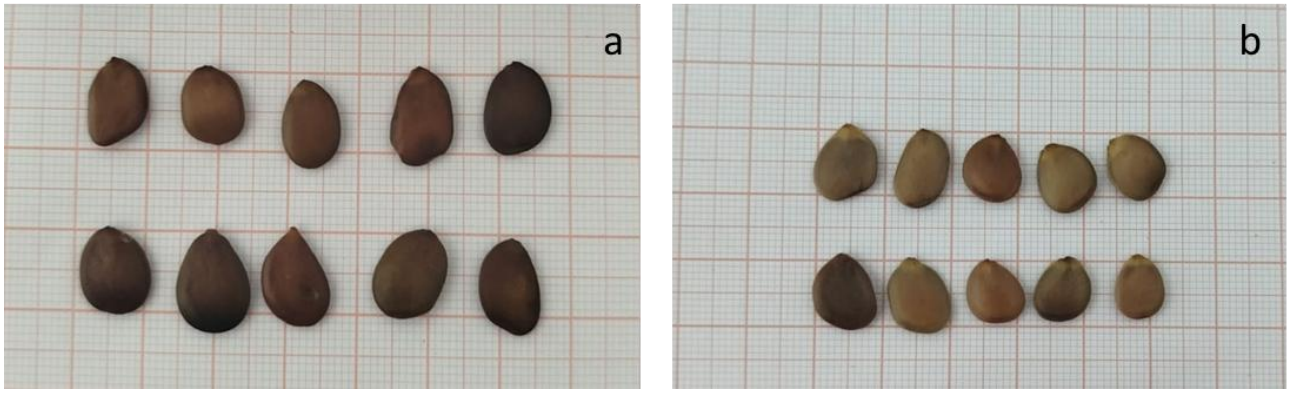


**Figure 1.** Seed morphology of *C. pyramidale* (a) and *C. microphyllum* (b)

Both images are shown at the same scale to highlight size differences. Scale bar = 5 mm.

The morphometric characteristics of *C. pyramidale* and *C. microphyllum* seeds are described in Table 2. In relation to Area (cm²) variable, *C. pyramidale* seeds are larger and more variant than *C. microphyllum* seeds. For Perimetry (cm), Height (cm), and Width (cm) variables the species have similar seed shape. Circularity (0.0-1.0), Roundness (0.0-1.0), and Solidity (0.0-1.0) differ between the two species, *C. pyramidale* is always larger in all variables. *C. microphyllum* seeds exhibit a more regular and uniform shape, yielding more homogeneous results and values that are more centered regarding data dispersion. *C. pyramidale* has greater variability in seed size and larger mean values.

**Table 2.** Morphometric measurements for variables: Area (AREA, mm²), Perimeter (PER, mm), Height (HEIG, mm), and Width (WIDT, mm) –as well as shape indices such as Circularity (CIR, 0.0-1.0), Roundness (ROUN, 0.0-1.0), and Solidity (SOL, 0.0-1.0)– for *Cenostigma pyramidale* and *Cenostigma microphyllum* seeds

| **Species** | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***Cenostigma pyramidale*** | | | | | | | ***Cenostigma microphyllum*** | | | | | | |
| **Descriptive statistics results / Variables analysed** | **AREA** | **PER** | **HEIG** | **WIDT** | **CIR** | **ROUN** | **SOL** | **AREA** | **PER** | **HEIG** | **WIDT** | **CIR** | **ROUN** | **SOL** |
| Minimum | 0.13 | 2.88 | 0.71 | 0.68 | 0.08 | 0.63 | 0.37 | 0.56 | 2.82 | 0.78 | 0.81 | 0.57 | 0.58 | 0.92 |
| Maximum | 1.48 | 5.22 | 1.61 | 1.43 | 0.93 | 0.95 | 0.97 | 1.33 | 4.75 | 1.46 | 1.36 | 0.93 | 0.91 | 0.97 |
| Mean | 0.94 | 3.7 | 1.18 | 1.05 | 0.86 | 0.79 | 0.95 | 0.89 | 3.59 | 1.13 | 1.05 | 0.87 | 0.8 | 0.95 |
| Stand. error | 0.02 | 0.04 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.03 | 0.01 | 0.01 | 0.01 | 0.01 | 0 |
| Variance | 0.04 | 0.17 | 0.03 | 0.02 | 0.01 | 0.01 | <0.01 | 0.02 | 0.12 | 0.02 | 0.02 | <0.01 | <0.01 | 61.33 |
| Stand. dev | 0.21 | 0.41 | 0.16 | 0.14 | 0.09 | 0.07 | 0.06 | 0.15 | 0.34 | 0.13 | 0.13 | 0.05 | 0.06 | 0.01 |
| Median | 0.93 | 3.69 | 1.18 | 1.02 | 0.87 | 0.79 | 0.96 | 0.89 | 0.36 | 0.11 | 0.1 | 0.88 | 0.81 | 0.96 |
| 25th percentile | 0.8 | 3.42 | 1.05 | 0.96 | 0.84 | 0.74 | 0.95 | 0.78 | 0.33 | 0.11 | 0.96 | 0.86 | 0.76 | 0.95 |
| 75th percentile | 1.07 | 3.98 | 1.3 | 1.12 | 0.89 | 0.82 | 0.96 | 0.98 | 0.38 | 1.24 | 1.12 | 0.89 | 0.83 | 0.96 |
| Skewness | −0.07 | 0.46 | −0.04 | 0.52 | −0.7 | 0.12 | −0.99 | 0.38 | 0.39 | −0.17 | 0.5 | −0.34 | −0.66 | −0.19 |
| Kurtosis | 0.15 | 0.76 | −0.13 | 0.54 | 0.6 | −0.16 | 0.98 | 0.46 | 0.85 | −0.08 | <0.01 | 0.14 | 0.78 | 0.57 |
| Coeff. var | 22.55 | 11.18 | 13.87 | 12.96 | 10.32 | 9.19 | 6.19 | 17.28 | 9.57 | 11.65 | 12.11 | 6.06 | 7.64 | 8.2 |

The morphometric characteristics of *C. pyramidale* and *C. microphyllum* seeds were described in Figure 1. There is one variable with the most marked differences, like Circularity variable, showing clearly distinct values between the groups. Some variables show very similar values between species, indicating low discriminatory power.

The results of the Principal Component Analysis are presented in Figure 3. The PC1 has the highest eigenvalue (0.19), explaining 65.42% of the total variance. The PC2 contributes an additional 23.46% to the explained variance, resulting in a cumulative 88.88% of the total variance accounted for by the first two components. These results suggest that PC1 and PC2 together are highly representative of the dataset, effectively summarizing its multivariate structure. The black color is used for *C. microphyllum* results and they are faster than *C. pyramidale* in blue color.

With regard to the Simpson's Dominance and Diversity Indexes (1 - D) and the Shannon Diversity Index, *C. pyramidale* and *C. microphyllum* seeds were described in Table 3. There is a low dominance between them (Simpson's Diversity Index), a high diversity and evenness (Simpson's Dominance Index), and an overall high diversity, rich and well-distributed in terms of species and abundance (Shannon Diversity Index).

Pearson correlation coefficients are described in Table 4. The overall size variables (Area, Perimeter, Height, and Width variables) form a strongly correlated group, while the shape indices (Circularity, Roundness, and Solidity) constitute another group. The variables within each group are significantly or strongly correlated with one another. Shape variables help distinguish features beyond size, making them useful for structural differentiation.

**Table 3.** Simpson's Dominance and Diversity Indexes (1 - D) and Shannon Diversity Index for variables: Area (AREA, mm²), Perimeter (PER, mm), Height (HEIG, mm), and Width (WIDT, mm) –as well as shape indices such as Circularity (CIR, 0.0-1.0), Roundness (ROUN, 0.0-1.0), and Solidity (SOL, 0.0-1.0)– for *Cenostigma pyramidale* and *Cenostigma microphyllum* seeds

| Index | AREA | PER | HEIG | WIDT | CIR | ROUN | SOL |
|---|---|---|---|---|---|---|---|
| Dominance_D | 5.206 | 5.055 | 5.085 | 5.078 | 5.036 | 5.061 | 0.01 |
| Simpson_1-D | 9.948 | 9.949 | 9.949 | 9.949 | 995 | 9.949 | 0.99 |
| Shannon_H | 5.277 | 5.293 | 5.29 | 5.291 | 5.293 | 5.292 | 4.605 |

**Table 4.** Pearson Correlation to variables: Area (AREA, mm²), Perimeter (PER, mm), Height (HEIG, mm), and Width (WIDT, mm) –as well as shape indices such as Circularity (CIR, 0.0-1.0), Roundness (ROUN, 0.0-1.0), and Solidity (SOL, 0.0-1.0)– for *Cenostigma pyramidale* and *Cenostigma microphyllum* seeds

| | AREA | PER | HEIG | WIDT | CIR | ROUN | SOL |
|---|---|---|---|---|---|---|---|
| **AREA** | | 0.84494 | 0.78207 | 0.75973 | 0.17269 | −0.12877 | 0.3698 |
| **PER** | 0.84494 | | 0.71228 | 0.65966 | −0.37308 | −0.13692 | −0.12307 |
| **HEIG** | 0.78207 | 0.71228 | | 0.23137 | 0.034921 | −0.18259 | 0.25649 |
| **WIDT** | 0.75973 | 0.65966 | 0.23137 | | 0.099708 | −0.019299 | 0.23891 |
| **CIR** | 0.17269 | −0.37308 | 0.034921 | 0.099708 | | 0.041624 | 0.82482 |
| **ROUN** | −0.12877 | −0.13692 | −0.18259 | −0.019299 | 0.041624 | | 0.01495 |
| **SOL** | 0.3698 | −0.12307 | 0.25649 | 0.23891 | 0.82482 | 0.01495 | |

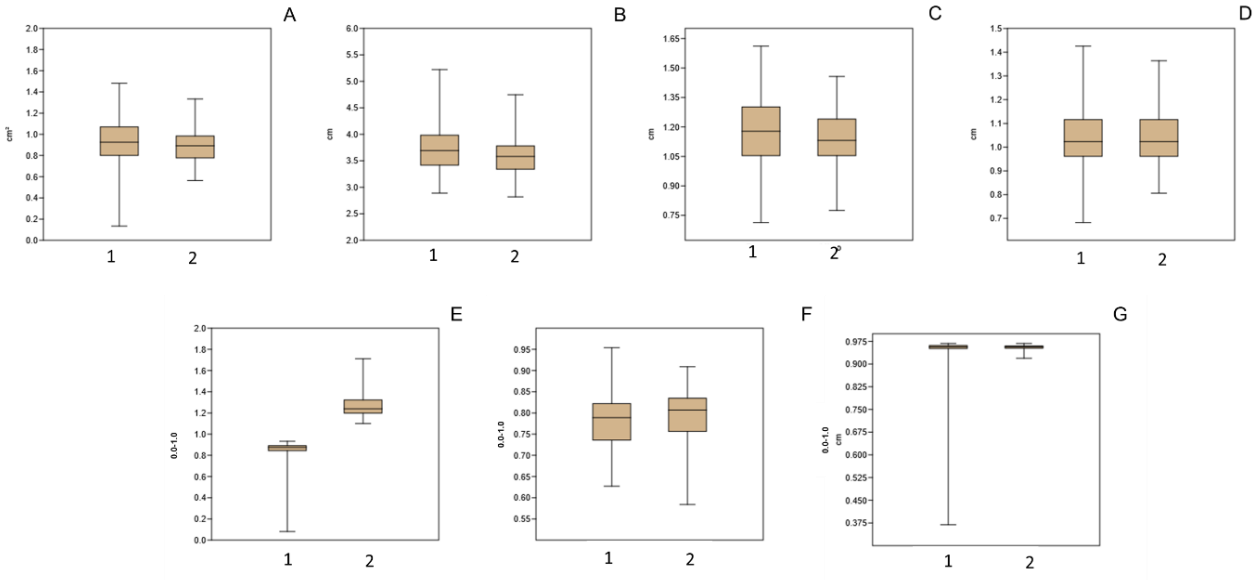


**Figure 2.** Box-plot to variables: Area (A - AREA, mm²), Perimeter (B - PER, mm), Height (C - HEIG, mm), and Width (D-WIDT, mm) –as well as shape indices such as Circularity (E - CIR, 0.0-1.0), Roundness (F - ROUN, 0.0-1.0), and Solidity (G - SOL, 0.0-1.0)–, for *Cenostigma pyramidale* (1) and *Cenostigma microphyllum* (2) seeds

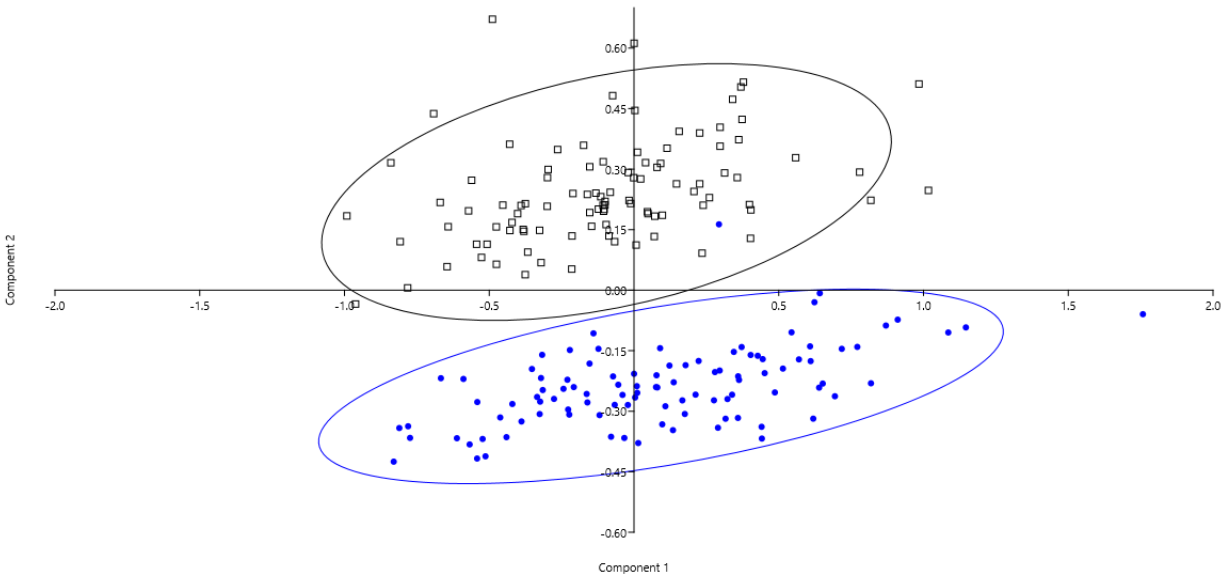


**Figure 3.** PCA to variables: Area (A - AREA, mm²), Perimeter (B - PER, mm), Height (C - HEIG, mm), and Width (D-WIDT, mm) –as well as shape indices such as Circularity (E - CIR, 0.0-1.0), Roundness (F - ROUN, 0.0-1.0), and Solidity (G - SOL, 0.0-1.0)– for *Cenostigma pyramidale* (blue) and *Cenostigma microphyllum* (black) seeds

## 4. Discussion

The forest diversities of the Caatinga regions are immense in terms of species richness, endemism, and strategies for survival and dispersion of seeds (Albuquerque et al., 2024). The characteristics of this environment make it unique with respect to the adaptive possibilities found within it, especially when compared with the surrounding vegetation formations (Arraes et al., 2024). Among the endemic species of this rich biodiversity are *Cenostigma pyramidale* and *Cenostigma microphyllum*.

*Cenostigma pyramidale* is an important species for the ecological balance of Caatinga environments, with widespread distribution throughout this territory. Studies conducted in the municipality of Sumé, in the state of Paraíba, have reported that this species shows remarkable plasticity regarding morphotypes and phenology under varying environmental conditions (Silva et al., 2024). Furthermore, the ability to monitor the diversity of its multiple uses by the local population and its survival conditions has proven relevant, making this one of the most extensively studied species of the Caatinga areas (Vilela et al., 2021).

The seed diversity of *C. pyramidale* described in the data presented in this research (Tables 1, 2, 3 and 4, Figures 1, 2 and 3) reveals the important phenotypic potential of this species, as reported by Amaro et al. (2023). Similar characteristics have been described regarding its tolerance to salinity (Nóbrega et al., 2024) and its response to hydration and dehydration cycles (W. Oliveira et al., 2023). The diversity observed in this study is comparable to that described by Patrício and Trovão (2020).

The seed diversity of *C. microphyllum* described in the data presented in this research (Tables 1, 2, 3 and 4, Figures 1, 2 and 3) represents an important milestone for the botany of the Caatinga areas. This is because the species is often easily confused with *C. pyramidale* and is sometimes neglected in studies (Gomes et al., 2019). According to W. Oliveira et al. (2023), this species is relevant in Caatinga areas, including for the recovery of degraded lands, although it is not resistant to high levels of aridity, probably due to its smaller size and lighter coloration, which favors desiccation (H. Oliveira et al., 2023). However, it presents potential for the recovery of degraded areas due to its capacity to associate with beneficial microorganisms and respond to inoculation (Gomes et al., 2019).

Variation in diaspore size in a dry forest can be an important factor in describing its genetic variability, as reported by Patrício and Trovão (2020), who highlight the significance of this factor in Caatinga areas. Based on the data described in Tables 2 and 3, we can observe a diversity in seed sizes of the two sampled species, confirming this local variability. These differences in diaspore size can facilitate dispersal to other areas, enhance resistance to inhospitable environmental conditions, and serve as factors influencing seed selection regarding dormancy, germination, and vigor within a population (Arraes et al., 2024; Patrício & Trovão, 2020).

This plasticity is thus an important survival and vigor strategy for seeds of *C. pyramidale* and *C. microphyllum* under the diverse conditions found in Caatinga areas, as shown in Tables 1, 2, 3 and 4.

Nonetheless, it is important to emphasize, in agreement with the writings of Aecyo et al. (2025) and Castro et al. (2024), that both of the cited species may undergo hybridization, and this factor is crucial for future research concerning the genetic and phenotypic classification of these species. The diversity *of C. pyramidale* and *C. microphyllum* in Caatinga areas is still underexplored (Arraes et al., 2024; Patrício & Trovão, 2020).

In this sense, further studies using computational tools for measuring seed size and morphological qualities could enhance species visualization and separation. Among these tools, the use of morphological and morphometric markers is possible, as performed in this research using seed images, as described by Loddo et al. (2023), Felix, Demartelaere, et al. (2023), Felix, Kratz, et al. (2023), and Felix et al. (2018, 2024).

Seeds of Caatinga species have already been described using image analysis, such as *Handroanthus impetiginosus* (Pimenta et al., 2024) and *Jatropha mollissima* (Walter et al., 2024), and this approach is recommended for the identification of native and forest species (Felix, Demartelaere, et al., 2023; Felix, Kratz, et al., 2023; Felix et al., 2018, 2024).

The high discriminatory power achieved through Area and Circularity parameters provides a practical diagnostic tool for identifying *Cenostigma* species, a task often hindered by morphological similarities in the field. As noted by Silva et al. (2024), understanding the phenological and morphological nuances of *C.*

*pyramidale* is essential for conservation efforts in the semi-arid region of Paraíba. By operationalizing these morphometric signatures, seed banks can implement high-throughput digital screening to ensure lot purity, reducing the taxonomic uncertainty that often hampers large-scale ecological restoration projects in the Caatinga biome.

Furthermore, integrating image-based analysis with physiological data, such as the reserve mobilization patterns discussed by Pereira-da-Silva et al. (2024), enables a more comprehensive characterization of seed quality. The ability to distinguish *C. pyramidale* from *C. microphyllum* with high statistical confidence ($p < 0.001$) supports the development of automated taxonomic keys. This approach not only refines the management of endemic forest resources but also aligns with modern seed technology protocols, offering a non-destructive, low-cost alternative for species authentication in biodiversity hotspots (Felix, Demartelaere, et al., 2023; Felix, Kratz, et al., 2023; Felix et al., 2018, 2024).

The automated use of imaging technologies for native species can be an efficient method for species characterization without the need for extensive biochemical or genetic testing (Felix, Demartelaere, et al., 2023; Felix, Kratz, et al., 2023; Felix et al., 2018, 2024), highlighting the importance of such approaches for a more refined analysis of forest seed diversity (Aecyo et al., 2025; Arraes et al., 2024; Castro et al., 2024; Patrício & Trovão, 2020). Consequently, the diversity of a forest would be directly linked to the diversity of its seeds, as affirmed by Patrício and Trovão (2020). Based on the clear morphometric segregation observed through PCA and clustering (Figure 2 and 3), we recommend the inclusion of seed shape descriptors in taxonomic keys and restoration seed banks for Caatinga species (Aecyo et al., 2025; Arraes et al., 2024; Castro et al., 2024; Felix, Demartelaere, et al., 2023; Felix, Kratz, et al., 2023; Felix et al., 2018, 2024; Patrício & Trovão, 2020).

## 5. Conclusions

*Cenostigma pyramidale* and *Cenostigma microphyllum* exhibit similar morphological characteristics, but show significant differences in seed shape and size variability. Morphometric indices, combined with multivariate statistical analysis, allowed for a clear distinction between the two species.

Morphological and morphometric characterization proved to be an efficient and reliable approach for interspecific differentiation. This study presents the first comparative analysis of seed morphology and morphometry of *C. pyramidale and C. microphyllum* using digital image analysis.

The findings support the use of morphometric parameters as consistent tools for species discrimination, with direct applications in ecological restoration, seed technology, and biodiversity conservation in the Caatinga areas.

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## Transparency of Data

Available data: The entire data set that supports the results of this study was published in the article itself.

## Use of Artificial Intelligence

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## Author Contribution Statement

| | TC Ferreira | EM Silva |
|---|---|---|
| Conceptualization | ■ | ■ |
| Data curation | ■ | |
| Formal analysis | ■ | |
| Funding acquisition | ■ | |
| Investigation | ■ | ■ |
| Methodology | ■ | ■ |
| Project administration | ■ | |
| Resources | ■ | |
| Software | ■ | |
| Supervision | ■ | |
| Validation | ■ | |
| Visualization | ■ | |
| Writing – original draft | ■ | |
| Writing – review and editing | ■ | |

## References

Abt, M., Loibl, K., Leuders, T., Van Dooren, W., & Reinhold, F. (2025). Understanding student errors in comparing data sets with boxplots. *Educational Studies in Mathematics, 120*(1), 169-193. https://doi.org/10.1007/s10649-025-10387-z

Aecyo, P., Costa, L., Jacobina, U. P., Harand, W., Leal, I. R., Souza, G., & Pedrosa-Harand, A. (2025). Integrative evidence on the hybridization between *Cenostigma microphyllum* and *C. pyramidale* (Leguminosae) in the Caatinga dry forest. *Plant Biology (Stuttgart)*, *27*(5), 749-760. https://doi.org/10.1111/plb.13753

Albuquerque, I. M. C. de, Rocha, B. de F., & Melo, J. I. M. de. (2024). Flora da Paraíba, Brasil: Loasaceae. *Hoehnea*, *51*, Article e1152023. https://doi.org/10.1590/2236-8906e1152023

Amaro, M. J. S., Silva, M. A. D. da, Ferraz, G. X. L., & Batista, E. V. (2023). Caracterização biométrica de sementes de *Cenostigma pyramidale* (Tul.) Gagnon & G. P. Lewis, coletadas em diferentes anos. In J. K. B. Andrade (Ed.), *Estudos e tendências atuais em Ciências Ambientais e Agrárias* (pp. 70-79). Editora Licuri. https://doi.org/10.58203/Licuri.21887

Arraes, J. C. de C., Velozo, C. de O., Vieira, F. J., & Conceição, G. M. da. (2024). Diversidade vegetal e da síndrome de seus diásporos (frutos e sementes) apontam maior relação por dispersão abiótica com áreas menos conservadas na Caatinga piauiense, NE Brasil. *Revista Brasileira de Gestão Ambiental e Sustentabilidade, 11*(29), 1105-1122. https://doi.org/10.21438/rbgas(2024)112904

Bezabih, G., Wale, M., Satheesh, N., Fanta, S. W., & Atlabachew, M. (2023). Forecasting cereal crops production using time series analysis in Ethiopia. *Journal of the Saudi Society of Agricultural Sciences, 22*(8), 546-559. https://doi.org/10.1016/j.jssas.2023.07.001

Castro, N., Mata-Sucre, Y., Carvalho-Sobrinho, J., Marques, A., Queiroz, R. T. de, & Souza, G. (2024). Genomic stability in *Cenostigma* Tul., (Caesalpinioideae, Fabaceae): Causes and consequences. *Botanical Journal of the Linnean Society, 204*(2), 137-151. https://doi.org/10.1093/botlinnean/boad043

Divi, S., Rao, G. D., & Anand, S. (2024). Understanding the impact of agriculture insurance: Insights and challenges of PMFY scheme from four states of India using pearson correlation. *Research on World Agricultural Economy*, *6*(1), 452-464. https://doi.org/10.36956/rwae.v6i1.1362

Felix, F. C., Castro, M. L. de L., Souza, W. M. A. T. de, Ferrari, C. dos S., & Pacheco, M. V. (2018). Biometria de sementes de espécies florestais da Caatinga por meio da análise de imagens. *Informativo ABRATES, 28*(1), 46-50.

Felix, F. C., Demartelaere, A. C. F., Medeiros, J. A. D. de, Pacheco, M. V., & Pereira, M. D. (2023). A condição climática de origem das árvores afeta a incidência de fungos e a qualidade de sementes de *Pityrocarpa moniliformis*? *Ciência Florestal*, *33*(2), Article e71378. https://doi.org/10.5902/1980509871378

Felix, F. C., Kratz, D., Ribeiro, R., & Nogueira, A. C. (2023). Characterization and differentiation of forest species by seed image analysis: A new methodological approach. *Ciência Florestal*, *33*(3), Article e73427. https://doi.org/10.5902/1980509873427

Felix, F. C., Kratz, D., Ribeiro, R., & Nogueira, A. C. (2024). Machine learning in the identification of native species from seed image analysis. *Journal of Seed Science*, *46*, Article e202446002. https://doi.org/10.1590/2317-1545v46277554

Gomes, S. E. V., Oliveira, G. M. de, Araujo, M. do N., Seal, C. E., & Dantas, B. F. (2019). Influence of current and future climate on the seed germination of *Cenostigma microphyllum* (Mart. ex G. Don) E. Gagnon & G. P. Lewis. *Folia Geobotanica, 54*, 19-28. https://doi.org/10.1007/s12224-019-09353-4

Leitão, J. R., Cunha, M. do C. L., & Ferreira, T. C. (2024). Morfologia de sementes, plântulas e tirodendros em espécies de Fabaceae ocorrentes no bioma Caatinga. *Pesquisa Florestal Brasileira*, *44*, Article e202102219. https://doi.org/10.4336/2024.pfb.44e202102219

Loddo, A., Di Ruberto, C., Vale, A. M. P. G., Ucchesu, M., Soares, J. M., & Bacchetta, G. (2023). An effective and friendly tool for seed image analysis. *The Visual Computer, 39*, 335-352. https://doi.org/10.1007/s00371-021-02333-w

Ma, Z. S. (2025). Measuring microbiome diversity and diversity-scaling analysis with Hill numbers. In M. Nagarajan (Ed.), *Metagenomics* (2nd ed., pp. 157-188). Academic Press. https://doi.org/10.1016/B978-0-323-91631-8.00012-3

Nóbrega, J. S., Silva, L. G. da, Silva, F. E. da, Santos, M. E. M., Silva, T. I. da, Bruno, R. de L. A., Lima, G. S. de, & Soares, L. A. dos A. (2024). Seed osmopriming with salicylic acid on induction of tolerance of *Cenostigma pyramidale* to water deficit. *Revista Árvore*, *48*, Article e4802. https://doi.org/10.53661/1806-9088202448263630

Oliveira, H., Pereira, S., & Santos, M. G. (2023). *Cenostigma microphyllum* seedlings in semiarid region grow faster under arbuscular mycorrhizal symbiosis, regardless of water availability. *Journal of Arid Environments, 212*, Article 104962. https://doi.org/10.1016/j.jaridenv.2023.104962

Oliveira, W., Cruz-Neto, O., Silva, J. L. S., Tabarelli, M., & Lopes, A. V. (2023). Aridity mediates the effect of wood extraction on the reproductive output of an endemic disturbance-adapted woody species (*Cenostigma microphyllum*, Leguminosae) in the Caatinga dry forest. *Austral Ecology*, *48*, 251-265. https://doi.org/10.1111/aec.13265

Patrício, M. da C., & Trovão, D. M. de B. M. (2020). Seed biometry: Another functional trait in caatinga. *Acta Scientiarum. Biological Sciences, 42*(1), Article e51183. https://doi.org/10.4025/actascibiolsci.v42i1.51183

Pereira-da-Silva, P., Andrade-Sousa, G., Penha-de-Oliveira, D. A., Campos-Silva, M., Alves-de-Oliveira, D. F., & Voigt, E. L. (2024). Discontinuous hydration changes reserve mobilization during seed germination in the Caatinga native species *Cenostigma pyramidale*. *New Forests, 55*(6), 1899–1912. https://doi.org/10.1007/s11056-024-10066-6

Pimenta, J. M. A., Souza, W. M. A. T. de, Egito, R. H. T. do, Ferrari, C. dos S., & Pacheco, M. V. (2024). Divergência fenotípica de *Handroanthus impetiginosus* por meio de imagens digitais. *Pesquisa Florestal Brasileira*, *44*, Article e202102257. https://doi.org/10.4336/2024.pfb.44e202202257

Secretaria de Defesa Agropecuária. (2013). *Instruções para análise de sementes de espécies florestais*. MAPA.

Silva, J. A. da, Bezerra, J. E. F., Gomes, A. C., Barbosa, F. M., & Lacerda, A. V. de. (2024). Phenological behavior of *Cenostigma pyramidale* (Tul.) E. Gagnon & G.P. Lewis in an area of Caatinga in the semi-arid region of Paraíba. *Brazilian Archives of Biology and Technology*, *67*, Article e24230723. https://doi.org/10.1590/1678-4324-2024230723

Vilela, L. M. B, Santos-Silva, C. A. dos, Roldan Filho, R. S., Araújo, S. de S., Ferreira-Neto, F. R. C., Oliveira, W. D. de, Amorim, L. L. B., Pandolfi, V., & Benko-Iseppon, A. M. (2021). *Cenostigma pyramidale*: Ethnomedicinal properties and perspectives on a legume tree highly adapted to semiarid 'Caatinga' region. In M. Rai, S. Bjattarai, & C. M. Feitosa (Eds.), *Ethnopharmacology of wild plants* (pp. 218-233). CRC Press. https://doi.org/10.1201/9781003052814-13

Walter, L. S., Felix, F. C., Nogueira, A. C., Pifano, D. S., Silva, M. A. da, & Kratz, D. (2024). Seed and seedling morphological characterization of *Jatropha mollissima* (Pohl) Baill. (Euphorbiaceae): A potential species with multiple uses. *Revista Ceres*, *71*, Article e71009. https://doi.org/10.1590/0034-737X2024710009