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# Physical and morpho-biometric characterization of fruits and seeds of the tree species Copaifera arenicola (Ducke)

# Caracterização física e morfobiométrica de frutos e sementes da espécie arbórea *Copaifera* arenicola (Ducke)

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#### Abstract

Knowledge of physical-morphological attributes of plant diaspores contributes to correct description, conservation and optimized agronomic use of the species. The objective of the study was to perform the physical and morpho-biometric characterization and establish biometric correlations of fruits and seeds of Copaifera arenicola. For the study, the internal-external morphology and the physical-biometric characterization of fruits and seeds were analyzed. The results were subjected to descriptive statistical analysis and Pearson's linear correlation. The fruit is an obovoid, dry, dehiscent and monospermic unilocular follicle, reddish-brown in color, with length of 19.92-33.58 mm, width of 13.24-28.5 mm, and diameter of 11.81-19.01 mm. The seed is spherical/ovoid, with length of 9.6-17.39 mm, width of 7.83-12.79 mm, diameter of 7.88-12.59 mm, and weight of 0.48-1.43 g. The colors of the testa, tegmen, cotyledon and aril of the seed are opaqueblack, dark red, cream-white and strong vellow, respectively. Thousand-seed weight is 854.6 g (1,170 seeds.kg<sup>-1</sup>), with a water content of 10.2%. The seeds fall into the small (9-11.8 mm), medium (11.8-14.60 mm) and large (14.6-17.4 mm) classes in individual size. The strong correlations of the variable seed volume index with seed weight (r=0.952; p=0.001), seed length (r=0.867; p=0.001) and seed width (r=0.861; p=0.001) indicated characteristic shape. The morphology of the C. arenicola fruit involved structures of adaptation to the environmental conditions of the Caatinga and of increase in the distribution capacity of the species. The seeds have homogeneous morphological attributes, with variation only in terms of biometric variables.

**Keywords**: Endemic of the Caatinga. Diaspores. Morphology. Classification. Correlation.

#### Resumo

O conhecimento de atributos físico-morfológicos de diásporos vegetais contribui para a descrição correta, para a conservação e uso agronômico otimizado da espécie. O objetivo do estudo foi realizar a caracterização física e morfobiométrica e correlações biométricas de frutos e sementes de Copaifera arenicola. Para o estudo, foi analisado a morfologia interna-externa e a caracterização físico-biométrica de frutos e sementes. Os resultados foram submetidos à análise estatística descritiva e correlação linear de Pearson. O fruto é um folículo oboval, seco, deiscente e unilocular monospérmico; com coloração marron-avermelhada; comprimento de 19,92-33,58 mm, largura 13,24-28,5 mm e diâmetro de 11,81-19,01 mm. A semente é esférica/ovoide, com comprimento de 9,6-17,39 mm, largura de 7,83-12,79 mm, diâmetro de 7,88-12,59 mm e peso de 0,48-1,43 g. A coloração da testa, do tegma, do cotilédone e do arilo da semente é preta-opaco, vermelha-escuro, creme-esbranquiçado e amarelo-forte, respectivamente. O peso de mil sementes é de 854,6g (1.170 sementes.kg<sup>-1</sup>), com teor de água de 10,2%. As sementes se enquadram nas classes pequena (9-11,8 mm), média (11,8-14,60 mm) e grande (14,6-17,4 mm) em tamanho individual. A forte correlação da variável índice de volume das sementes com peso das sementes (r=0,952; p=0,001); com comprimento das sementes (r=0,867; p=0,001) e com largura das sementes (r=0,861; p=0,001), indicaram forma característica. A morfologia do fruto de C. arenicola envolveu estruturas de adaptação às condições ambientais da Caatinga e de aumento da capacidade de distribuição da espécie. As sementes apresentam atributos morfológicos homogêneos, com variação apenas quanto as variáveis biométricas.

Palavras-chave: Endêmica da Caatinga. Diásporos. Morfologia. Classificação. Correlação.

#### 1 Introduction

Seed quality goes beyond germination capacity, being influenced by physical attributes, such as water content, weight, size, shape, and texture of the seed coat, which vary between species (Dobrzański; Stępniewski, 2013; Almeida *et al.*, 2014; Souza *et al.*, 2014; Coxon; Longstaff; Burns, 2019). In view of this, there is the morphological polymorphism of seeds, evidenced by the shape, size and heterogeneous compositions, which is more accentuated in native species under wild conditions, contrasting with the uniformity observed in domesticated or improved seeds (Matilla; Gallardo; Puga-Hermida, 2005; Pedrini *et al.*, 2020; Vieira *et al.*, 2021).

Seed size and shape, in particular, are important quantitative characteristics, and knowledge of these parameters allows a better understanding of ecological processes, such as longevity, seed bank behavior, and dispersal mechanisms (Bekker *et al.*, 2002; Wang; Ives, 2017). This knowledge also helps in the use of biotechnological methods for germplasm conservation, stock management and improvement of reproductive practices, promoting uniformity in emergence and improvement of traits (Carvalho; Nakawaga, 2012).

In the context of commercial production, morphological variations of seeds represent challenges for forestry, especially in relation to classification, storage, and handling, in addition to the difficulty of flowability in mechanical sowing (Pedrini *et al.*, 2020), as well as knowledge of the physical characteristics of seeds, such as water content, which is essential for the identification of the species, and processes such as drying, storage and sowing (Bekker *et al.*, 2002; Matilla *et al.*,

2005; Dobrzański; Stępniewski, 2013; Samoylichenko; Mokiychuk, 2023). In view of this, the selection of seeds based on these attributes influences their behavior and germination success (Almeida *et al.*, 2014; Matilla *et al.*, 2005).

Among the species of the Brazilian flora, angiosperms produce seeds of different shapes and sizes, generally little known (Duarte *et al.*, 2016). Environmental conditions influence these seeds, especially in native forest species of the Caatinga, which have polymorphism, including variations in size, color, and attached structures (Meiado *et al.*, 2012; Dantas, 2014). This plant diversity is reflected in the 3,347 phanerogamic species cataloged in the Caatinga, of which 15.72% (n=526) are endemic (Fernandes; Queiroz, 2018; Fernandes; Cardoso; Queiroz, 2019). Among these, *Copaifera arenicola* (Ducke) J.Costa & L.P.Queiroz stands out, a tree species of the Fabaceae family, with occurrence restricted to semi-arid climate environments, associated with sandy soils of deep to very deep sedimentary origin, dystrophic and well drained (Cardoso; Queiroz, 2007; Gama; Nascimento Júnior, 2019; Brasil, 2020).

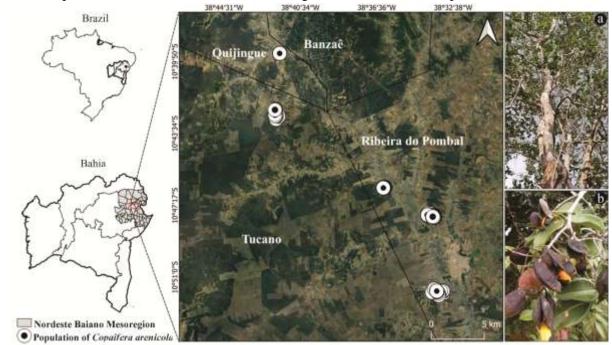
In this context, the present study aims to perform the physical and morpho-biometric characterization of fruits and seeds, in addition to determining the presence of correlations between the biometric attributes to meet the needs of classification and technological application of *Copaifera arenicola* (Ducke) J.Costa & L.P.Queiroz seeds.

#### 2 Material and Methods

### 2.1 Collection, processing and storage

Fruits and seeds of *Copaifera arenicola* (Ducke) J. Costa & L. P. Queiroz were collected between August and October 2023, from 116 different plants, distributed in five natural populations located in Caatinga remnants in the municipalities of Ribeira do Pombal, Banzaê, Tucano and Quijingue, located in the *Nordeste Baiano* Mesoregion of Bahia (Figure 1). The selection criteria for the matrices, such as size, health and age of the trees, were based on Piña-Rodrigues; Figliotia; Silva (2015).

**Figure 1.** Location of the populations of *Copaifera arenicola* (Ducke) J. Costa & L. P. Queiroz plants distributed in Caatinga remnants in the *Nordeste Baiano* Mesoregion of Bahia: (a) profile of an adult specimen of *C. arenicola* and (b) fruiting in *C. arenicola* with ripe dehiscent fruits.



The diaspore collection region includes remnants of Carrasco-type Caatinga vegetation, within the Caatinga Phytogeographic Domain (Moro *et al.*, 2016; Fernandes; Queiroz, 2018), with shrub-tree typology (Jesus *et al.*, 2018), located in a region with a semi-arid tropical climate (Sales *et al.*, 2023) of the Bsh type, characterized as dry and hot (Alvares *et al.*, 2013). The average annual rainfall is 711 mm, with an average annual temperature of 24.2 °C (Gama; Jesus, 2018). The soils of the collection region are of sedimentary origin, ranging from deep to very deep, sandy, dystrophic and with low water retention. *Neossolos Quartzarênicos* (Quartzipsamments) and *Latossolos* and *Argissolos Vermelhos-Amarelos* (Oxisols and Ultisols, respectively) prevail in the region (Cardoso; Queiroz, 2007; Gama; Nascimento Júnior, 2019; Brasil, 2023).

After collection, an exsiccata of the species (voucher: HURB35717) was deposited in the Herbarium of the Federal University of Recôncavo da Bahia, Cruz das Almas campus, and the material (fruits and seeds) was sent to the Seed Analysis Laboratory of the same University, where it went through the processing processes. Subsequently, the seeds were stored in a refrigerator (5.0  $\pm$  0.5 °C; 50  $\pm$  5% RH), packed in properly identified transparent polyethylene plastic bags.

# 2.2 Fruit and seed morphology

Morphological description of the fruit was carried out based on external aspects (fruit type, shape, texture and color of the pericarp, in addition to the number of seeds per fruit) and internal

aspects (presence and consistency of the mesocarp and endocarp) of fruits that were manually opened with a blade in the longitudinal position.

Morphological description of seeds was carried out considering their external structures (hilum position, presence of raphe, micropyle, and type, texture and color of the seed coat). For the internal description (type and position of the hypocotyl-radicle axis and presence, shape and color of the cotyledon), a sample of seeds was initially hydrated for 30 min. Then, the seeds were manually sectioned with a cutting blade, in three types of openings: longitudinal (parallel to the cotyledons), transverse to the middle (perpendicular to the longitudinal axis) and radial tegumentary/cotyledonary cut.

Description was carried out with the aid of a benchtop magnifying glass, using the terminologies of Souza (2006), Vidal and Vidal (2011) and Barroso, Morim and Ichaso (2012). The recorded morphological characteristics of fruits and seeds are presented on self-explanatory scaled boards, based on images obtained in a natural light environment, with the aid of a semi-professional camera.

Identification of the color of fruits and seeds was carried out with fresh diaspores after harvest, without any type of treatment, such as drying or conservation in 70° alcohol. The description was carried out visually, based on the color notation proposed by Ferguson (2014), using the Munsell Color System Chart, which is based on values of intensity, hue and chroma (Munsell, 2012).

## 2.3 Fruit and seed biometry

For the analysis of fruit morpho-biometry, 200 fruits were randomly selected. Biometric measurements were performed with a digital caliper (precision of 0.01 mm), obtaining fruit length (FL), width (FW) and diameter (FD). FL was measured considering the imaginary orthogonal line of the longitudinal axis, from the terminal apex to the base of insertion of the peduncle, FW considering the imaginary orthogonal line perpendicular to the length, and FD (thickness) considering the imaginary orthogonal line perpendicular to the length and width.

Biometric characterization of seeds was performed using samples from a single lot formed by the different seed populations aiming at a general characterization. Thus, 200 seeds were selected at random. A digital caliper (precision of 0.01 mm) was used to obtain the measurements of seed length (SL), width (SW) and diameter (SD). Weight (SWT) was measured using an analytical scale (0.001 g precision). Seed volume index (SVI) was calculated by the product of SL x SW x SD, and the basic density ( $\rho$ S) by the product (SWT / SVI) x 1000. SL was measured considering the imaginary orthogonal line of the longitudinal axis, from the hilum to the base, SW considering the

imaginary orthogonal line perpendicular to the length, and SD (thickness) considering the imaginary orthogonal line perpendicular to both length and width.

With the biometric measurements obtained, the shape (JS) and flattening (HS) coefficients of the seeds were calculated by the relationship of the biometric variables (Equation 1), based on the classification of Romero (1961). The JS coefficient was classified as: spherical/ovoid (1.16 to 1.42), elliptical (1.43 to 1.65), short oblong/reniform (1.66 to 1.85), medium oblong/reniform (1.86 to 2.00) or long oblong/reniform (> 2.00). The HS coefficient degree was classified as: flattened (< 0.69), semi-full (0.70 to 0.79) or full (> 0.80).

$$JS = \frac{SL}{SW}$$
;  $HS = \frac{SD}{SW}$ , (Eq. 1)

where: JS = coefficient of seed shape; SL = seed length (mm); HS = degree of seed flattening; SW = seed width (mm); SD = seed diameter (thickness) (mm).

The biometric analyses did not consider the differences by population, since they were carried out considering the single lot of seeds for general characterization.

# 2.4 Physical characterization and size classification of seed

Based on the Seed Analysis Standards (*Regras para Análises de Sementes* - RAS) (Brasil, 2009), thousand-seed weight (TSW), expressed in grams, was determined from the average weight of eight replicates of 100 seeds, considering a coefficient of variation lower than 4%. The number of seeds.kg<sup>-1</sup> was calculated from the TSW.

Seed water content was determined by the basic reference method, since these are species that contain volatiles (oils), as recommended by RAS. For this, some seeds were fragmented using pruning shears into small pieces ( $\leq 7.0$  mm) close to the weight (g) of five intact seeds. Subsequently, from the fragmented material, two replicates of  $4.5 \pm 0.5$  g were immediately collected and, after being weighed on an analytical scale (precision of 0.001 g), were distributed in metal capsules, identified and subjected to drying in an oven at a temperature of  $103 \pm 2$  °C for 17  $\pm 1$  h. Then, the dry mass was measured on an analytical scale. Moisture percentage was calculated based on wet weight, showing results within the tolerance of the amplitude range of 0.3 - 2.5% between the samples (Equation 2):

$$U\% = 100(Wi - Wf) / Wi - t,$$
 (Eq. 2)

where: Wi = initial weight (weight of the container and its lid plus the weight of the wet seed); Wf = final weight (weight of the container and its lid plus the weight of the dried seed) and t = tare weight (weight of the container with its lid).

The seeds were classified into three categories of uniform sizes (small, medium and large), based on the length measurement, according to the basic principle of seed separation by Vaughan, Gregg and Delouche (1976), using a random sample of 200 seeds. The size classes were established

by the class center of the distribution, with large seeds (LGS) included in the interval formed by the highest mean, small seeds (SMS) included in the interval of lowest mean, and medium seeds (MDS) included between the average lengths of the large and small seed classes. The hierarchy of classes was as follows: SMS < MDS < LGS.

# 2.5 Frequency distribution and Pearson's linear correlation

The values of the biometric measurements of the orthogonal axes (length, width and diameter), weight (specific mass), specific volume and calculated shape and flattening coefficients were analyzed by means of descriptive statistics and frequency distribution histogram. After verifying the normality of the errors by the Shapiro-Wilk test ( $p \le 0.05$ ), Pearson's linear correlation analysis was performed to determine the strength and direction of the correlations between the biometric variables of the seeds. The significance of the correlation coefficients (r) was tested using the t-statistic. A significance level of 5% was adopted in both analyses. The analyses were performed using R software, version 4.3.3 (R Core Team, 2024).

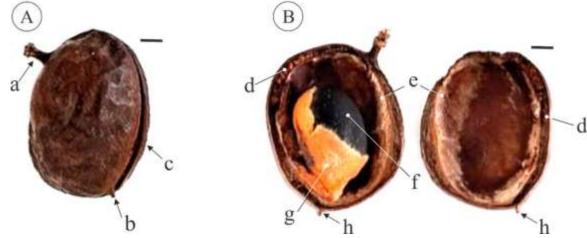
#### 3 Results and Discussion

# 3.1 Fruit and seed morphology

The fruit of the tree species *Copaifera arenicola* (Ducke) J.Costa & L.P.Queiroz is a short, non-friable legume, classified as an obovoid-shaped follicle, attenuated towards the base (Figures 2A and B). It is a dry, dehiscent fruit, with a woody and rigid consistency, bivalve, with a peduncle slightly inclined in relation to the longitudinal axis (a). It has a short rostrum (b), located in the apical position (h).

The pericarp of the ripe fruit has a wrinkled appearance, consisting of a woody mesocarp (d) growing next to the thick fibrous endocarp (e), although both are differentiated from each other. The fruit opens in the ventral position, in a single longitudinal valve (c).

**Figure 2.** Longitudinal profile of the fruit of the species *Copaifera arenicola* (Ducke) J.Costa & L.P.Queiroz closed (A) and open (B), with the presence of peduncle (a); rostrum (b and h); longitudinal valve (c); epicarp (d); mesocarp (e); seed (f) and aril (g). Bar = 2.5 mm.



The fruit is unilocular and monospermic, with seed in apical placentation and partially filled locule (f). It has a funicular-type aril, covering the seeds in different proportions, ranging from 1/3 to a little more than half (Figures 2Bg and 3Aa).

From the morphological description of the fruit of *Copaifera arenicola* (Ducke) J.Costa & L.P.Queiroz, classified, according to Barroso, Morim and Ichaso (2012), as a follicle, it was possible to observe that this type of legume is common to all species of the genus *Copaifera* L., as indicated in Rigamonte-Azevedo, Wadt and Wadt (2004) and Costa (2017). The morphological description of the fruit of *C. arenicola* in the present study corroborates that obtained by Costa (2017), although the color of the valves, considered by the author as vinaceous yellow, was not observed in this study.

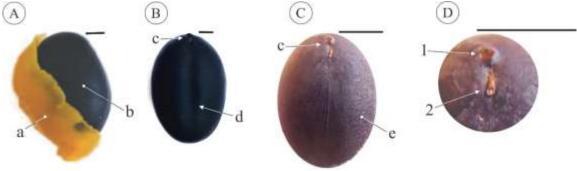
The fruit, with a wrinkled texture and reddish-brown color, opens spontaneously through a univalve ventral cleft, releasing the seeds, which confirms that it is a dehiscent dry fruit in its last stage of maturation, evidenced during seed collection. Barroso, Morim and Ichaso (2012) clarified that this opening occurs by mechanical action, resulting from the change in position of the fibers of the pericarp tissues due to their slow desiccation, breaking the tissue that longitudinally joins the valves and, thus, exposing the seed to dispersal. According to Souza (2006), the tissue present in the ventral suture between the margins of the carpel is less fibrous, which facilitates the separation and opening of the fruit, which is why the follicles are formed by a single carpel, according to the author.

It is also worth noting the presence of an appendage, clearly visible on the ripe fruit, called the rostrum, located in the opposite position to the peduncle. This persistent structure, according to Barroso, Morim and Ichaso (2012), is the stylet of the pistil of the flower and contributes as a facilitating element to the identification of the species.

It was observed that, in dehiscence, the seed remains attached to the fruit by the aril still in the parent plant for a long time. This may be one of the mechanisms of attraction of dispersers, due to both the strong yellowish color of the aril and the odor of the fruit/aril, resulting from the presence of coumarin, a secondary metabolite common in fruits of this genus (Rigamonte-Azevedo; Wadt; Wadt, 2004; Franco et al., 2021). This phenomenon can be confirmed for *C. arenicola*, since Fenner and Thompson (2009) stated that seeds dispersed by mammals usually produce aromatic substances, while those dispersed by birds usually have bright and striking colors. Authors such as Gama and Monteiro Júnior (2019) observed the dispersal of *C. arenicola* seeds carried out by birds and small primates directly from the tree canopy in a Caatinga remnant in Bahia. The aril in the seed of *C. arenicola* also served as a reward for the maned wolf (*Chrysocyon brachyurus* Illiger) and *Atta laevigata* ants (F. Smith, 1858) in their dispersal, as recorded by Fagundes et al. (2022) in the Caatinga region in the extreme north of Minas Gerais. The ecological importance that this structure represents for the zoochoric syndrome of the genus *Copaifera* L. was reported by Rigamonte-Azevedo, Wadt and Wadt (2004), especially for its nutritional value, being rich in lipids. Thus, the diaspore effect of *C. arenicola* is characterized.

The external morphology of the seed revealed a tegument with a glabrous, hard and uniform black color testa, with matte texture (b) (Figure 3). The hilum, positioned on the apical axis (c), does not have a halo. In the abaxial-longitudinal position (d) of the seed, there is a mild depression, forming a fissure line. On an enlarged scale (C, D), it was observed that the texture of the testa is rough, with conspicuous streaks (e), and the hilum is circular, opaque, small and protruding (c). The hilum (1) is adjacent to the rudimentary ventral raphe in the shape of a ridge (2), with conspicuous micropyle.

**Figure 3.** Lateral-longitudinal profile (A), frontal-longitudinal profile (B and C) and frontal-apical profile (D) of the seed of the species *Copaifera arenicola* (Ducke) J.Costa & L.P.Queiroz with presence of aril (a), matte black color (b), hilum (c) and (1), abaxial fissure line (d), wrinkled textured testa (e) and conspicuous micropyle (2). Bar = 2.5 mm.



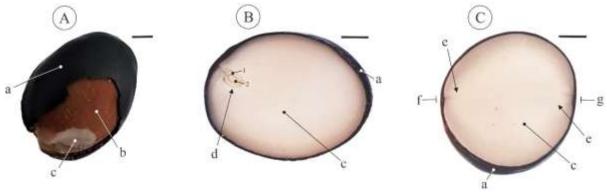
**Source:** Authors.

The hard integument in the seed, described in *C. arenicola*, generally plays a protective role against damage to the embryo, as observed by Bareke (2018), and against ingestion by dispersing animals, according to Schmidt (2007). According to Fenner and Thompson (2009), hardness is generally associated with physical dormancy. Thus, the hardness in *C. arenicola* seed, added to the dispersal syndromes, can be a crucial factor for success in the establishment of the species in environments far from the parent plant, promoting the formation of abundant populations with uniform spatial distribution, as observed by Gama *et al.* (2020). In addition, this dispersal can reduce the possibility of inbreeding, since seeds are transported away from their parents, as found by Carvalho *et al.* (2010) in a study on the genetic diversity of *Copaifera langsdorffii* Desf. in a remnant of Atlantic Forest in São José do Rio Preto, SP.

Aril color also plays an important phytophysiological role in identifying the preferred habitat of the species. Contrary to the statement of Costa (2017), who suggested that the *Copaifera* L. species that occur in open and sunnier environments of the Caatinga are shrubby in size and have a white aril, *C. arenicola* in these environments has an arboreal size and an aril of strong yellow color. This demonstrates that species of the genus *Copaifera* L. have varied suitability, still little studied in the diverse environmental complex of the Caatinga. Finally, in addition to zoochoric dispersal, Rigamonte-Azevedo, Wadt and Wadt (2004) also mentioned the barochoric syndrome, resulting from the action of gravity, common to the genus *Copaifera* L. The species *C. arenicola* has this syndrome, although it does not occur immediately after the fruit is opened.

In radial profile (A), it was observed that the seed has two integuments, with the testa markedly distinct (a) from the tegmen (b), which constitutes the internal integument, under which there are two fleshy, smooth and plane-convex cotyledons, arranged in parallel (Figure 4). In a longitudinal section (B), with one of the cotyledons removed (c), it was possible to observe that the hypocotyl-radicle axis was invaginated, crypto-radicular, small, straight and parallel to the cotyledons (d). Skirting the hypocotyl-radicle axis (2), there is an emarginate base (1). In cross-section (C), it was possible to observe a natural fissure separating the cotyledons in their longitudinal axis, connecting the adaxial position (f) to the abaxial position (g).

**Figure 4.** Radial profile (A), longitudinal section (B) and cross-section (C) of the seed of the species *Copaifera arenicola* (Ducke) J.Costa & L.P.Queiroz with presence of testa (a), tegmen (b), cotyledon (c), hypocotyl-radicle axis (d) and (2), emarginate base (1), natural fissure (e) of the adaxial (f) and abaxial (g) position towards the longitudinal axis of the seed. Bar = 2.5 mm.



Based on Munsell's (2012) color system and Ferguson's (2014) nomenclature, fruits in their last stage of maturation have a reddish-brown color (Munsell-value: 5YR 4/4, reddish-brown). The seeds have cream-white cotyledons (Munsell-value: 5Y 9/1, pale yellowish-white / whitey yellow) and integument with opaque black testa and dark red tegmen (Munsell-value: 10R 3/4: red-dusky), covering the entire length of the seed. The aril shows a strong yellow color (Munsell-value: 5Y 8/10, strong-yellow).

Another prominent morphological attribute in the species are the fleshy cotyledons, which, in addition to serving as functional reserves for embryo development during germination (Schmidt, 2007; Fenner; Thompson, 2009), also promote dispersal by leaf-cutting ants *Atta opaciceps* Borgmeier, 1939. Attracted by these structures, the ants transport the cotyledons to their nests, spreading them along the way, as observed by Gama *et al.* (2019).

# 3.2 Fruit and seed biometry and frequency

The specific weight of the seed ranged from 0.48 to 1.43 g, with an average of 0.91 + 0.22 g. The average measurements of seed length (SL), width (SW) and diameter (SD) were 13.29 $\pm$ 1.49 mm, 9.68 $\pm$ 1.07 mm and 9.97 $\pm$ 0.81 mm, respectively. The mean coefficient of the shape value (JS) and the degree of flattening (HS) was 1.38 $\pm$ 0.13 (classified as spherical/ovoid: JS < 1.42) and 1.04 $\pm$ 0.11 (classified as full seed: HS > 0.80). For the seed volume index (SVI) and seed basic density ( $\rho$ S), the mean values were 1,301 $\pm$ 327.01 mm³ and 0.701 $\pm$ 0.054 g.cm⁻³. Regarding fruit measurements, the average length (FL) was 24.48 $\pm$ 2.29 mm, width (FW) was 17.09 $\pm$ 2.28 mm, and diameter (FD) was 14.58 $\pm$ 1.28 mm (Table 1).

**Table 1.** Descriptive statistics for the set of biometric variables of fruits and seeds of *Copaifera arenicola* (Ducke) J.Costa & L.P.Queiroz.

	Seeds							Fruits			
Variables	SWT	SL	SW	SD	SVI	ρS	JS	HS	FL	FW	FD
	(g)	(mm)	(mm)	(mm)	$(mm^3)$	(g.cm <sup>-3</sup> )		пэ	(mm)	(mm)	(mm)
Moda	0.78	12.85	8.99	10.09*	1109.65*	0.693*	1.37*	1.08*	24.48*	17.09*	14.58*
Median	0.86	13.16	9.49	10,00	1212.67	0.703	1.37	1.06	24.77	17.69	14.34
Mean	0.91	13.29	9.68	9.97	1301.45	0.706	1.38	1.04	25.12	18.07	14.42
$SD(\pm)$	0.22	1.49	1.07	0.81	327.01	0.054	0.13	0.11	2.29	2.28	1.28
Minimum	0.48	9.60	7.83	7.88	649.88	0.472	1.09	0.65	19.92	13.24	11.81
Maximum	1.43	17.39	12.79	12.59	2055.24	0.869	1.74	1.27	33.58	28.50	19.01
Amplitude	0.95	7.79	4.96	4.71	1405.36	0.397	0.65	0.62	13.66	15.26	7.20
Skewness	0.62	0.31	0.59	0.22	0.59	-0.55	0.64	-0.76	0.58	1.21	0.52
Kurtosis	-0.43	0.04	-0.20	0.62	-0.59	4.52	0.49	0.84	0.40	2.32	0.21
CV	24.50%	11.20%	11.10%	8.10%	25.10%	7.62%	9.20%	10.30%	9.10%	12.60%	8.90%

Where: SWT = specific weight of the seed; SL = seed length; SW = seed width; SD = seed diameter; SVI = seed volume index;  $\rho$ S = seed basic density; JS = coefficient of the seed shape value; HS = coefficient of the degree of seed flattening; FL = fruit length; FW = fruit width; FD = fruit diameter; SD( $\pm$ ) = standard deviation; CV = coefficient of variation. (\*) There is more than one mode, and only the mode with the highest density estimate was reported.

Source: Authors.

Using herbarium material, Costa (2017) found for *C. arenicola*, mean values of 12.00 mm for seed length (SL) and 10.0 mm for width (SW), which are mean values approximately similar to the results found within a significant range. The results reveal a variation between the extremes in a wide dimensional polymorphism, with amplitude ranging from 0.95 for weight (SWT) to 7.7 for SL.

For Fenner and Thompson (2009), morphometric variations in seeds of wild species are a direct consequence of the genetic constitution of the parent plant and its physiological effect in different parental environments. In addition, under the influence of seasonal variations and environmental conditions, these variations generate changes in seed structure and composition, according to Dobrzański and Stępniewski (2013). Fenner and Thompson (2009) also suggest that this variation in seed size may be an inevitable consequence of resource constraints, which limit the ability of the parent plant to control individual seed size.

On the other hand, also for Fenner and Thompson (2009), the variation in the size of seeds from different parental environments expands the range of conditions under which the plant can germinate, increasing the chances of reproduction in unpredictable environments.

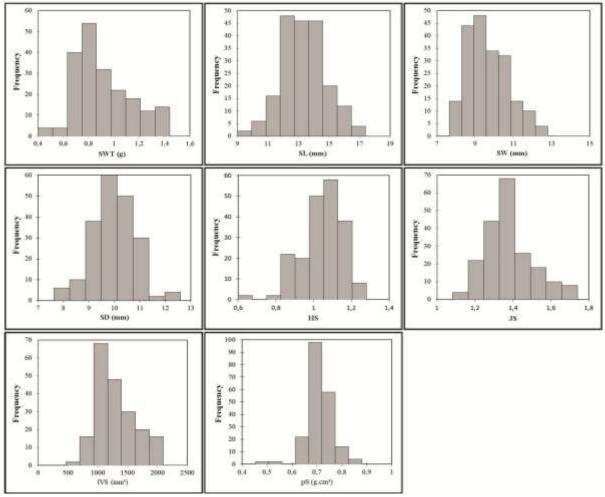
Since *C. arenicola* fruits are predominantly monospermic, fruits containing two juxtaposed and malformed seeds have been found in some cases, since the biometric characteristics of well-formed seeds, described as spherical/ovoid, full, average weight (SWT) of 0.91 g and average

density ( $\rho$ S) of 0.71 g.cm<sup>-3</sup>, are strongly opportune to barochoric dispersal, favoring seed drop at the right time.

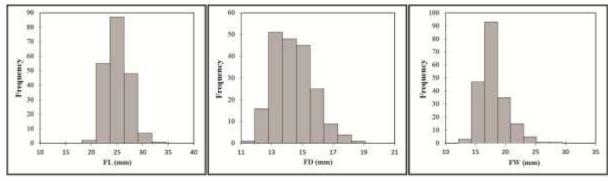
Regarding the biometric variables obtained for *C. arenicola* fruits, average length (FL) of 25.12 mm, ranging from 19.92 to 33.58 mm, and an average width (FW) of 18.07 mm, ranging from 13.24 to 28.50 mm, are values close to those found by Costa (2017) in herbarium data, with FL ranging from 23.00 to 33.00 mm and FW ranging from 19.00 to 27.00 mm. In the present study, it was seen that the results showed a variation in the biometric values, with less discrepancy (more homogeneous sizes) compared to the seeds.

The frequency distribution was approximately normal ( $p \ge 0.05$ ) for all sets of seed and fruit variables. Based on Pearson's skewness coefficient, the distribution indicates that the dataset of all biometric variables of seeds and fruits showed positive and moderate skewness ( $g_1<1.0$ ), with the distribution of the data concentrated more to the left, except for the variables  $\rho S$  and HS in seeds, which concentrated more to the right (negative skewness). A high degree of skewness ( $g_1>1.0$ ) was verified only for FW among the fruit variables. Based on kurtosis, the distribution of the variables  $\rho S$  and FW occur in a more closed manner (Table 1, Figures 5 and 6).

**Figure 5.** Frequency distribution of biometric variables weight (SWT), length (SL), width (SW) and diameter (SD), degree of flattening (HS), shape value coefficient (JS), volume index (SVI) and basic density ( $\rho$ S) of seeds of the species *Copaifera arenicola* (Ducke) J.Costa & L.P.Queiroz.



**Figure 6.** Frequency distribution of the biometric variables length (FL), diameter (FD) and width (FW) of fruits of the species *Copaifera arenicola* (Ducke) J.Costa & L.P.Queiroz.



Source: Authors.

The frequency distribution with positive skewness observed in all seed variables indicates a greater concentration of data on the left, with mode and median values lower than the mean. This

shows a predominance of seeds in medium to small size classes, while larger sizes occur less frequently.

The exception was observed in the values of the degree of flattening (HS) and density ( $\rho$ S), which group a greater number of large seeds (values above the mean) in these parameters. In addition, the frequency curve for the variable  $\rho$ S showed high kurtosis, with a significant number of extremely high values, reflecting less dispersion of the data around the mean. These results suggest a striking characteristic of the species: the investment in fuller (less flattened) seeds, which are predominantly grouped in medium to small sizes and with a higher frequency of high density.

# 3.3 Physical characterization and classification of seed

Regarding the physical characterization, the water content (U) of the seed, based on wet weight, was 10.2%. Thousand-seed weight (TSW) was 854.6 g, which suggests that one kilogram of *C. arenicola* seeds contains approximately 1,170 seeds.

Regarding the size class, the seeds were classified as large (LGS) when they were within the interval of highest mean, represented by the center of the class (16.00 mm) within the frequency range (14.60 mm - 17.40 mm), small (SMS), in the interval of the lowest center of class (10.40 mm), and medium (MDS), representing 70% of the sampled seeds, with intermediate lengths between the large and small seed classes (Table 2).

**Table 2.** Size classes based on length measurement in seeds of the species *Copaifera arenicola* (Ducke) J.Costa & L.P.Queiroz.

Classes	Size interval (mm)	Center of class (mm)	Frequency		
Small Seeds (SMS)	9.00   11.80	10.40	24	12.0%	
Medium Seeds (MDS)	11.80   14.60	13.20	140	70.0%	
Large Seeds (LGS)	14.60   17.40	16.00	36	18.0%	

Source: Authors.

As for the water content (U) found for *C. arenicola* seed, of 10.2% on a wet weight basis, it classifies the seed as intermediate in relation to desiccation tolerance. In this context, Silva (2019) states that seeds with this physiological behavior do not tolerate desiccation to lower levels, as they cannot maintain viability for long periods. Souza Júnior and Brancalion (2016) explained that seeds dispersed with water content below 15% can be classified as intermediate and orthodox, tolerating drying and low temperatures, which allows their storage for long periods. Almeida *et al.* (2014) highlighted that the storage of seeds with water content between 10 and 13% is acceptable under conditions of conservation in an open environment, being tolerable for six to eight months. Bewley *at al.* (2013) pointed out that each seed species withstands a minimum water content, below which

the deterioration process can be initiated or accelerated, since moisture content and temperature are factors that influence the longevity of the seeds during storage, as also pointed out by Bewley *at al.* (2013).

According to the criterion established by the Seed Analysis Standards (RAS) (Brasil, 2009), the thousand-seed weight (TSW) obtained, of 854.6 g in *C. arenicola*, with approximately 1,170 seeds per kilogram, indicates seeds of large size, as it is above 200 g, the reference value considered by RAS.

Although TSW is an important parameter to assess the state of maturity and health of the seed lot, in addition to determining the appropriate number of seeds per package for storage (Brasil, 2009), the predetermined analysis standards described in RAS do not suit most seeds of forest species. As a result, the differentiation of the specific size per unit of seed into size classes offers important practical utility, and serving even as a key piece to integrate the effects of plant coexistence and improve understanding on the subject, according to Dylewski *et al.* (2020).

In this context, it is noted that most of the seeds (70%), grouped as individual units in a medium-size class, between 11.80 and 14.60 mm in length, may represent a strategy of the parent plant to increase the chances of survival by producing medium-sized seeds more frequently. This corroborates the statement of Fenner and Thompson (2009), who explained that, in a specific environment, there is a single optimal seed size. Below this optimum, a small increase in size provides great benefits in terms of suitability; above optimum, further increases in seed mass bring progressively lower returns.

In view of this, authors such as Dobrzański and Stępniewski (2013) emphasized the importance of estimating characteristics such as shape, size, volume, and specific mass of seeds with greater precision, considering them fundamental parameters for other approaches and technological applications. However, in native forest seeds, there is a deficiency in classification or even the absence of a measurement standard, which, according to Coxon *et al.* (2019), occurs due to the lack of widespread recognition of their usefulness. Thus, although a varied production of seed sizes may be a stable evolutionary strategy for the plant (Fenner; Thompson, 2009), from the perspective of silviculture, it is not a desirable production. Authors such as Pontes *et al.* (2018) reported that morphological variation in seeds of native species is a problem, as it hinders processing and application operations. In addition, high failure rates in seed-based restoration, as reported by Pedrini *et al.* (2020), were attributed to factors related to variation in seed size, which result in incorrect handling.

### 3.4 Pearson's linear correlation

Pearson's linear sample correlation ( $p \le 0.05$ ) was significant for all relationships between variables, except for the correlation of JS with SWT, SD, SVI and  $\rho$ S, as well as of  $\rho$ S with SWT, SL and HS. Strongly positive correlations were obtained for all associations with SVI (r = 0.95 with SWT; r = 0.87 with SL; r = 0.86 with SW), in addition to the correlations between SWT and SL (r = 0.86) and SWT and SW (r = 0.81), Table 3.

**Table 3.** Pearson's linear correlation ( $p \le 0.05$ ) between the biometric variables: length (SL), width (SW), diameter (SD), volume index (SVI), shape value coefficient (JS), degree of flattening (HS) and basic density ( $\rho$ S) of seeds of the species *Copaifera arenicola* (Ducke) J.Costa & L.P.Queiroz.

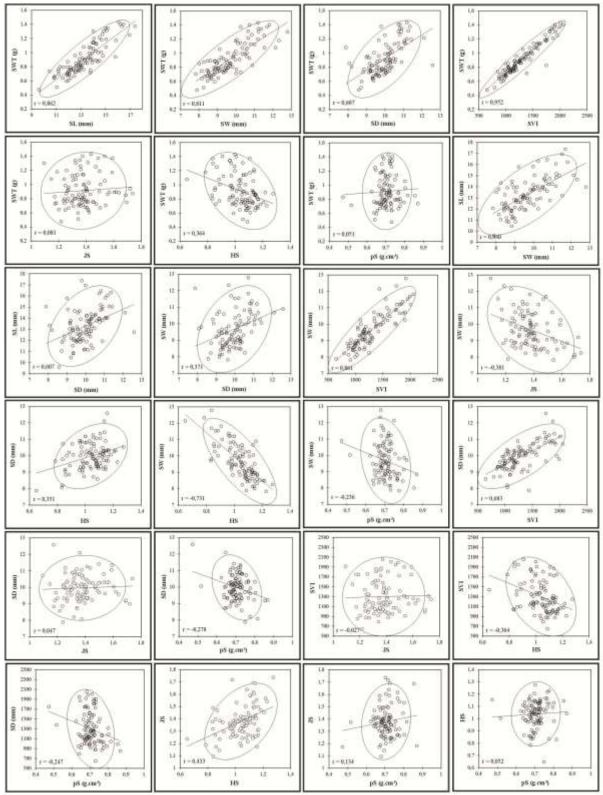
Variables	SWT	SL	SW	SD	SVI	JS	HS
SL	r = 0.862*						
	(p = 0.001)	_					
SW	r = 0.811*	r = 0.664*	_				
	(p = 0.001)	(p = 0.001)					
SD	r = 0.607*	r = 0.407*	r = 0.371*				
	(p = 0.001)	(p = 0.001)	(p = 0.001)	_			
SVI	r = 0.952*	r = 0.867*	r = 0.861*	r = 0.683*			
	(p = 0.001)	(p = 0.001)	(p = 0.001)	(p = 0.001)			
JS	$r=0.081^{\mathrm{ns}}$	r = 0.431*	r = -0.381*	$r=0.047^{\mathrm{ns}}$	$r=0.027^{\mathrm{ns}}$		
	(p = 0.255)	(p = 0.001)	(p = 0.001)	(p = 0.513)	(p = 0.703)	_	
HS	r = -0.364*	r = -0.362*	r = -0.731*	r = 0.351*	r = -0.364*	r = 0.433*	
	(p = 0.001)	(p = 0.001)	(p = 0.001)	(p = 0.001)	(p = 0.001)	(p = 0.001)	
ρS	$r = 0.051^{ns}$	$r = -0.125^{ns}$	r = -0.236*	r = -0.278*	r = -0.247*	$r = 0.134^{ns}$	$r = 0.052^{ns}$
	(p = 0.473)	(p = 0.077)	(p = 0.001)	(p = 0.001)	(p = 0.001)	(p = 0.059)	(p = 0.465)

Where: \* = significant and  $^{\text{ns}} = \text{non-significant}$ .

**Source:** Authors.

Pearson's linear sample correlations ( $p \le 0.05$ ) can be observed in the scatter plots, which are characterized by the correlation coefficient (r) (Figure 8).

**Figure 8.** Scatter plot of Pearson's linear correlation data ( $p \le 0.05$ ) between the biometric variables, length (SL), width (SW), diameter (SD), volume index (SVI), shape value coefficient (JS), degree of flattening (HS) and basic density ( $\rho$ S) of seeds of the species *Copaifera arenicola* (Ducke) J.Costa & L.P.Queiroz.



Pearson's linear correlation analysis revealed very strong and positive associations between seed weight (SWT) and seed length (SL), as well as between seed weight (SWT) e the seed width (SW), indicating an intense directly proportional synergy in the growth of these variables. On the other hand, the weak correlation between SWT and seed SD, although also positive, can be explained by the fact that SD reached its maximum growth more slowly compared to SL and SW, while these last-mentioned variables may have maintained a continuous and even simultaneous growth with that of SWT, due to the accumulation of dry matter in the seed, although this relationship is not causal.

On the other hand, the shape coefficient (JS) showed a weak and positive correlation with SL and an even lower and negative correlation with SW. This shows that the same parameters (SL and SW) used to obtain the dimensionless value of JS, cannot, individually, provide any conclusion regarding the spherical/ovoid shape of the seed. Regarding the degree of flattening (HS), there was a strong and negative correlation with SW and a weak and positive correlation with SD. This pattern shows that the less flattened (fuller) the seed, the greater its width, since the width is related to the dimension perpendicular to the diameter. Thus, the fuller it is, the higher the SD.

Very strong and positive correlations were also observed between SVI and the variables SWT, SL and SW, while the correlation with SD was weak. The correlation of SVI with SWT, the strongest of all, confirms the intense affinity between these two parameters (volume and mass), a relationship that implies a considerable basic density ( $\rho$ S), of approximately 0.71 g.cm<sup>-3</sup>. For this reason, the correlation of SWT with  $\rho$ S was very weak, since density is a direct ratio between SWT and SVI, as observed in the coefficients of JS and HS.

On the other hand, although the correlation and the scatter plot between biometric variables do not prove, according to Martins and Rodrigues (2014), the existence of a cause-and-effect relationship, they determine the strength with which the linear relationship between two variables occurs, predicting when they vary together (Asuero; Sayago; González, 2006). Thus, the use of correlation as a selection criterion in seeds becomes important, since the strength of the association must be considered when replacing one biometric variable with another.

Thus, it is possible to consider that the different populations may keep among themselves variations that could affect seed biometry due to environmental influence, and there may be some direct relationship between biometric variables and seed germination viability. These observations entail a possible and opportune direction for future investigations.

## **4 Conclusions**

The morphology of the fruit and seeds of *Copaifera arenicola* involves structures adapting to the environmental conditions of the Caatinga and increasing the distribution capacity of the species.

The presence of aromatic and showy arils, considerable specific weight and fleshy cotyledons in the seeds of *C. arenicola* establish ecological importance with different zoochoric and barochoric dispersal syndromes.

*C. arenicola* seeds have a spherical/ovoid shape, with high variability in the biometric parameters, which indicates a non-conformity, as they are not homogeneous.

The physiological behavior of *C. arenicola* seeds, regarding their tolerance to desiccation, is of the intermediate type, based on the water content found, equal to 10.2%.

Thousand-seed weight (TSW) for *C. arenicola* is 854.6 g, which classifies its seeds as large, corresponding to approximately 1,170 seeds per kilogram.

The medium individual size of *C. arenicola* seeds, between 11.80 and 14.60 mm, predominates in terms of the number of seeds per most frequent class.

The seed volume index (SVI) variable, highly correlated with seed weight (SWT), length (SL) and width (SW) and the correlations of SWT with SL and SW, indicate that *Copaifera* arenicola seeds have a characteristic shape.

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