Morphological diversity and identification key for landraces of the Amerindian yam in central Amazon

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Abstract – The objective of this work was to select an optimal set of morphological descriptors, in order to characterize the phenotipical diversity of Amerindian yam (*Dioscorea trifida*) landraces cultivated in the municipality of Caapiranga, in the central Amazon region of Brazil, and to develop a botanical identification key for them. A collection of 140 accessions and an experimental plot with a representative sample of 20 landraces were used to test 64 morphological descriptors for the aerial and subterranean plant parts. Forty-eight descriptors were selected, of which 13 were for tubers, 12 for stems, 14 for leaves, and 9 for inflorescences and seed. A cluster analysis based on the morphological data showed the formation of two landrace groups with greater similarity: white and purple pulp tubers. The results provide tools for in situ and ex situ conservation and for plant breeding programs, considering the importance of maintaining and recognizing the value of Ameridian yam as an important native genetic resource for food security in the region.

Index terms: *Dioscorea trifida*, agrobiodiversity, Dioscoraceae, genetic resources, native food plant, phenotypic similarity.

Diversidade morfológica e chave de identificação para variedades locais de cará na Amazônia Central

Resumo – O objetivo deste trabalho foi selecionar um conjunto ideal de descritores morfológicos para caracterizar a diversidade fenotípica de variedades locais de cará (*Dioscorea trifida*) cultivadas no Município de Caapiranga, na região da Amazônia central, e desenvolver uma chave de identificação botânica. Uma coleção de 140 acessos e um cultivo experimental com uma amostra representativa de 20 variedades regionais foram utilizados para testar 64 descritores morfológicos para as partes aéreas e subterrâneas da planta. Foram selecionados 48 descritores, dos quais 13 para tubérculos, 12 para caules, 14 para folhas e 9 para inflorescências e sementes. Uma análise de agrupamento baseada nos dados morfológicos mostrou a formação de dois grupos de variedades locais com maior similaridade: tubérculos de polpa branca e de polpa roxa. Os resultados fornecem ferramentas para programas de conservação in situ e ex situ e de melhoramento, considerando-se a importância de manter e reconhecer o valor do cará como recurso genético nativo importante para a segurança alimentar na região.

Termos para indexação: *Dioscorea trifida*, agrobiodiversidade, Dioscoraceae, recursos genéticos, plantas alimentícias nativas, similaridade fenotípica.

Introduction

"Cará" (*Dioscorea trifida* L.) is a native Amazonian food plant and an important crop for food sovereignty and security. *D. trifida* is an herbaceous, climbing, perennial, dioecious, and allogamous plant, with a high intraspecific genetic diversity (Nascimento et al., 2013). There is evidence that its center of origin is the Amazon, where it shows autotetraploidy in the cultivated form, and tri- or diploidy in the wild form (Bousalem et al., 2010). The species has high nutritional value and medicinal properties (RamosEscudero et al., 2010; Teixeira et al., 2013). In other Brazilian regions, the most well known species for cultivation are *D. alata* and *D. cayennensis* or "caráda-costa" (Heredia Z. et al., 2000). Although important worldwide as food source, yams are neglected and underused crops (Hernández-Bermejo & León, 1994; Azevedo & Duarte, 1997).

The integration of agrobiodiversity conservation by small farmers with agricultural intensification has been considered as one of the main strategies for sustainability and food security to face global environmental and socioeconomic changes (Zimmerer, 2013). In the state of Amazonas, small-scale farmers maintain in their plantations local landraces of *D. trifida*, which may have favorable agronomic characteristics and high capacity to tolerate biotic and abiotic stresses. Farmers from Caapiranga reported that, in 2015, they produced 20-30 Mg ha⁻¹ of fresh tubers, and a production per pit around 5.9 to 8.8 kg. In Colombia, Acevedo Mercado et al. (2015) obtained an average yield of 30.6 Mg ha⁻¹ for *D. trifida*, and 27.0 Mg ha⁻¹ for *D. esculenta*, with 1x1 m spacing.

Castro et al. (2012) identified various local landraces of *D. trifida* in the main cultivation region in the municipality of Caapiranga, observing that some landraces are no longer cultivated, and that there are only reports by farmers about their existence. There has been an abandonment of their cultivation or a concentration of the production of only one species, or of few landraces, which can lead to a "genetic erosion" and the loss of the species diversity, which leads to the risk of extinction of landraces.

The knowledge on the morphological diversity of the species is essential to define the morphological descriptors for the characterization and management of genetic resources, as well as the use of the genetic variability for genetic improvement with multiple purposes. A standard yam descriptor list (Descriptors..., 1997; Norman et al., 2011) is available for assessing variation among Dioscorea species. There has been a considerable effort to assess morphological descriptors of some other Dioscorea, except for D. trifida (Rhodes & Martin, 1972; Onyilagha, 1986; Dansi et al., 1999; Islam et al., 2011). The only report for D. trifida is that of Nascimento et al. (2013), who used 12 morphological traits to described fifty-one accessions of D. trifida from the states of São Paulo, Santa Catarina, and Mato Grosso, and included only two accessions acquired at fairs in the Amazonas state. Neither list of descriptors nor identification key is available to describe D. trifida accessions.

The identification of easily detectable morphological characteristics of the aerial and underground parts would allow of the creation of an identification key to differentiate the landraces of *D. trifida*, providing a useful tool for in situ and ex situ agrobiodiversity conservation. The morphological description is also important for the differentiation of landraces with similar characteristics, and for the detection

of duplicate genotypes that might receive different nomenclatures in different places. Landraces with the same name may assume different morphological characteristics of the aerial part, which are often not considered by the farmers. Each area of cultivation contains a considerable diversity of landraces, making it necessary to define the specific or common characteristics of a landrace.

The objective of this work was to select an optimal set of morphological descriptors to characterize the phenotypical diversity of *D. trifida* landraces, cultivated in Caapiranga municipality, in the central Amazon region, and to develop an identification key.

Materials and Methods

The snowball sampling technique (Bernard, 2006) was used to identify farmers who cultivate different landraces of *D. trifida*. This technique allowed of the identification of informants who helped to locate the farmers and, from these, other possible farmers were indicated. In this way, we found 45 family farmers who cultivated different landraces. The differentiation of the landraces was done firstly by the popular names given by the farmers who cultivate them, and by the qualitative morphological characteristics clearly visible in the field, such as pulp color and shape of tubers, among other traits, which are also indicated by the local nomenclature.

In March 2015, we collected 140 accessions in the municipality of Caapiranga, accompanying the farmers in the cultivation areas. The tubers were harvested directly, or chosen when they were stored next to the cultivation area, under the shade of the forest, covered by palm leaves. Some accessions were obtained in the houses of the farmers, where they were stored in bags. Ten accessions were collected in the locality of Membeca, which is located in the municipality of Manacapuru, but only 32 km away from the city of Caapiranga by road. Castro et al. (2012) provide a complete description of the Caapiranga collecting sites. For comparison, an accession was collected in the metropolitan area of Manaus, in a commercial plantation of a Japanese descendant, and three other accessions were collected in farmers' markets, in two other municipalities: one accession in Humaitá, and two in Boa Vista do Ramos.

For the planting experiment, we selected 20 accessions that differ most morphologically (Table 1), to include purple and white colored tubers, and male and female plants, from a larger collection kept at the experimental farm of the Universidade Federal do Amazonas since 2013. The study has its approval by the Research Ethics Committee of the Universidade Federal do Amazonas (CAAE: 39407314.3.0000.5020), and participant farms signed informed consents. The locations of the accessions are presented in Figure 1. An experimental design with randomized blocks was adopted, with four plants per plot, and four blocks totaling 320 plants. The spacing distance between plants was 3 m, in order to ensure that lateral branches during growth did not reach nearby plants, thus, t there were no shading and no root contact between plants.

We chose an area without inclination, and uniform for soil and vegetation type, which was formerly a secondary forest. Trunks and roots of the trees were removed, and the soil was plowed. A composite soil sample was collected from 20 subsamples, at 1-20 cm soil depth, by the zigzag method, which was chemically analyzed by Embrapa Amazônia Ocidental (Table 2). The soil was classified as a Xanthic Haplustox, poor in organic matter, with low cation-exchange capacity, high acidity and high-aluminum content.

Soil correction and fertilization was done with 480 g m⁻² dolomite lime (25-30 % CaO e 15-20 % MgO) and 3 kg m⁻² dry and homogenized chicken manure. The surface soil of an area of 1 m² and 20 cm depth was mixed with the fertilizer and used to form the planting pit. For each landrace under homogenization criteria, healthy tubers of medium size of the same parent plant were chosen and stored for three weeks after harvest. The tubers were planted in the horizontal position, at approximately 15 cm soil depth in the pit.

The climbing support for each plant had a pyramidal form, and made with three bamboo sticks of 220 cm length crossed and fixed with wire. The aim of this system was to avoid contact between plants to facilitate the morphological description. During the first month, a formicide was applied to control the attack of leaf-cutting ants of the genus *Atta*. The vegetation between plants was cut, every two months, with a motorized brushcutter.

The morphological assessments were carried out in two phases, the first one during the rainy season because during this time there was availability of

Accession	Municipality	Community	Popular name	Cultivation area	Latitude	Longitude
1	Caapiranga	Maloca	Rabo-de-mucura	1	3°17'37.4"S	61°11'04.7"W
2	Caapiranga	Patauá	Branco-casquinha-roxa	2	3°18'17.2"S	61°11'47.5"W
3	Caapiranga	Lago de Arara	Casado	3	3°24'55.9"S	61°22'03.4"W
4	Caapiranga	Lago de Arara	Durão	3	3°24'55.9"S	61°22'03.4"W
5	Caapiranga	Lago de Arara	Boliviano	4	3°25'16.0"S	61°22'58.9"W
6	Caapiranga	Lago de Arara	Açaí-redondo	4	3°25'16.0"S	61°22'58.9"W
7	Caapiranga	Lago de Arara	Barbudo	5	3°24'51.5"S	61°22'08.6"W
8	Manacapuru	Membeca	Pé-de-burro	6	3°08'48.6"S	61°08'28.7"W
9	Manacapuru	Membeca	Ovo-de-cavalo	6	3°08'48.6"S	61°08'28.7"W
10	Caapiranga	Campinas	Açaí-comprido	7	3°18'47.7"S	61°06'59.5"W
11	Caapiranga	City	Mão-de-onça	8	3°19'47.2"S	61°12'37.8"W
12	Caapiranga	City	Branco-comprido	9	3°19'34.9"S	61°12'50.3"W
13	Caapiranga	Estrada Ary Antunes	Branco-redondo	10	3°17'02.4"S	61°12'52.9"W
14	Caapiranga	Estrada Ary Antunes	Pé-de-elefante	11	3°16'09.5"S	61°13'30.1"W
15	Caapiranga	Estrada Ary Antunes	Roxo-comum	12	3°17'42.4"S	61°12'49.1"W
16	Caapiranga	Estrada Ary Antunes	Macaxeira	13	3°17'59.5"S	61°12'41.6"W
17	Manaus	Colônia Japonesa	Japonês	14	3°04'18.0"S	59°59'13.8"W
18	Boa Vista do Ramos	City	Preto	15	2°58'11.1"S	57°35'26.5"W
19	Boa Vista do Ramos	City	Pé-de-porco	15	2°58'11.1"S	57°35'26.5"W
20	Humaitá	City	Roxo-de-humaitá	16	7°30'28.55"S	63°1'13.97"W

Table 1. Accessions of the Amerindian yam (*Dioscorea trifida*) selected for the experimental planting, with their respective origins, popular names, and geographical coordinates.

aerial parts, and the second one during the beginning of the dry season, when the tubers were harvested.

The Munsell color charts for plant tissues (Munsell Color, 1977) were used to assess differences in the tuber pulp pigmentation. An optimal set of descriptors that show best the unique characteristics of each landrace was used to create the identification key for the assessed landraces.

In order to perform a cluster analysis, we opted for the transformation of the morphological characteristics into binary data (presence, absence) (Bressan et al., 2011), which allowed of the similarity estimation through the Jaccard index. Based on the similarity matrix, a cluster analysis was applied by the UPGMA method (unweighted pair-group method using arithmetic mean) using the FDiversity statistical software program (Casanoves et al., 2011).

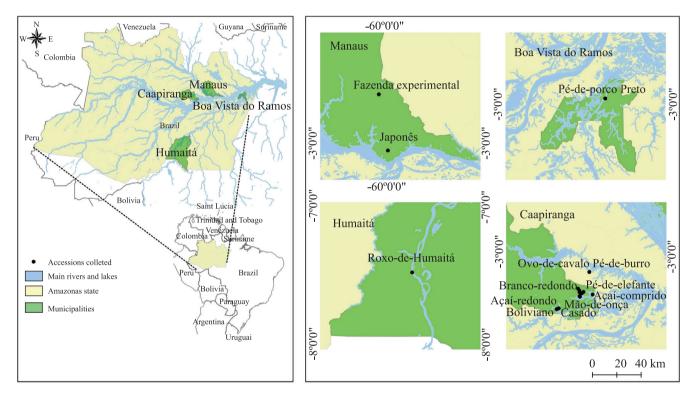


Figure 1. Collection sites of the evaluated accessions of Amerindian yam (*Dioscorea trifida*) in the municipalities of Manaus, Boa Vista do Ramos, Humaitá, and Caapiranga in the Central Amazon region of Brazil, according to the cartographic base of IBGE (2010).

Table 2. Results of the soil analysis of the experimental cultivation area of Amerindian yam (*Dioscorea trifida*) in the Central Amazon region of Brazil.

pН	С	SOM	Р	K	Na	Ca	Mg	Al	H+Al	SB	CECef	CECpot	BS	m	Fe	Zn	Mn	Cu
$\mathrm{H}_{2}\mathrm{O}$	(g	kg-1)	(1	mg dm	-3)				(cmol _c	dm-3)			(%	⁄0)		(mg c	lm ⁻³)	
4.1	19	32.7	2	17	2	0.1	0.1	1.9	6.17	0.3	2.2	6.5	4.4	87	138	0.46	1.1	0.2

C, organic carbon; SOM, soil organic matter; SB, sum of exchangeable bases; CECef, effective cation-exchange capacity; CECpot, cation-exchange capacity at pH 7; BS, base saturation index; m, aluminum saturation index.

Results and Discussion

The identified morphological differences facilitated the preparation and selection of 48 descriptors and the identification of the 20 landraces of *D. trifida*, with 13 descriptors for tubers, 12 for stems, 14 for leaves, and 9 for inflorescences and seed (Table 3). A subset of 16 descriptors used to identify species of Dioscorea (Descriptors..., 1997) was discarded after verification. These descriptors were not useful because they vary greatly within the same plant, or between plants of the same variety. This may be a result of high-genetic variability or environmental effects. The following descriptors were discarded: stems (number of main stems, number of wings, number of internodes up to the first branch, number of branches in the stem, and

Table 3. Morphological descriptors and characteristics for tubers, stems, leaves, flowers, and fruits of 20 landraces of Diocorea trifida.

wrinkles in the apical part, and poly-angular wrinkles in the distal partColor of the skinBrown; dark brown.Thickness of the skinVery thin, < 0.5mm; thin> 0.5mm.Color of the budsGreen; yellowish-green; light purple; dark purple.Capacity of buddingEasy; difficult (up to one month later).Tendency to branchAbsent; slight; moderate.Tuber shapeRound; oval and less oblong; oval; oval and very oblong; cylindrical; triangular, distal par wider; curved; apical part thin and long; wider than long; flat and irregular.Color of the tuber fleshWhite; yellowish-white; light purple; dark purple; white with a thin purple layer (≤ 1mm) the outermost cortex; white with purple spots; white with a purple outer layer of 0.5-1 cm	scriptors	Characteristics					
Presence of roots Absent; scarce; dense. Location of the roots All over the tuber uniformly; concentrated in the apical part. Roughness of the skin Smooth almost wrinkle free; medium roughness; deep wrinkles; two types of wrinkles (lin wrinkles in the apical part, and poly-angular wrinkles in the distal part Color of the skin Brown; dark brown. Thickness of the skin Very thin, < 0.5mm, thin> 0.5mm. Color of the buds Green; yellowish-green; light purple; dark purple. Capacity of budding Easy; difficult (up to one month later). Tendency to branch Absent; slight; moderate. Tuber shape Round; oval and less oblong; oval; oval and very oblong; cylindrical; triangular, distal part wider; curved; apical part thin and long; wider than long; flat and irregular. Color of the tuber flesh White; yellowish-white; light purple; dark purple; white with a thin purple layer of 0.5-1 cm purple apical part; purple with ight spots; purple with a white layer of 1-2 cm in the distal Uniformity of tuber flesh color Uniform; polycolored. Brownish oxidation of the tuber flesh after cutting Non browning; intensive browning; browning only in the exterior part or in some spots. Color of young stem > 30 cm Green; green with little purple pigmentation (PP). Color of mature stem (1 m height) Green; green, with little PP; green with much PP. <		Tubers					
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Tuber shapeRound; oval and less oblong; oval; oval and very oblong; cylindrical; triangular, distal par wider; curved; apical part thin and long; wider than long; flat and irregular.Color of the tuber fleshWhite; yellowish-white; light purple; dark purple; white with a thin purple layer (≤ 1mm) the outermost cortex; white with purple spots; white with a purple outer layer of 0.5-1 cm purple apical part; purple with light spots; purple with a white layer of 1-2 cm in the distal Uniformity of tuber flesh colorUniformity of tuber flesh colorUniform; polycolored.Brownish oxidation of the tuber flesh after cuttingNon browning; intensive browning; browning only in the exterior part or in some spots.Color of young stem > 30 cmGreen; green with little purple pigmentation (PP).Color of young stem wings >30 cmGreen; green with little PP; green with much PP.Presence of wings on the young stemPresent; absent.Color of mature stem (1 m height)Green; green with little PP; green with much PP.Color of mature stem (15 cm height)Green; green with little PP; green with much PP.Color of mature stem wings (15 cm height)Green; green with little PP; green with much PP.Color of mature stem wings (15 cm height)Green; green with little PP; green with much PP.Color of mature stem wings (15 cm height)Green; green with little PP; green with much PP.	pacity of budding	Easy; difficult (up to one month later).					
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the outermost cortex; white with purple spots, white with a purple outer layer of 0.5-1 cm purple apical part; purple with light spots; purple with a white layer of 1-2 cm in the distal Uniform; polycolored.Uniformity of tuber flesh color Brownish oxidation of the tuber flesh after cuttingUniform; polycolored.Brownish oxidation of the tuber flesh after cuttingNon browning; intensive browning; browning only in the exterior part or in some spots.Color of young stem > 30 cmGreen; green with little purple pigmentation (PP).Color of young stem wings >30 cmGreen; green with little PP; green with much PP.Presence of wings on the young stemPresent; absent.Color of mature stem (1 m height)Green; green with little PP; green with much PP.Color of mature stem wings (1 m height)Green; green with little PP; green with much PP.Color of mature stem (15 cm height)Green; brownish-green; green with little PP; green with much PP.Color of mature stem wings (15 cm height)Green; green with little PP; green with much PP.Color of mature stem wings (15 cm height)Green; green with little PP; green with much PP.	ber shape	Round; oval and less oblong; oval; oval and very oblong; cylindrical; triangular, distal part wider; curved; apical part thin and long; wider than long; flat and irregular.					
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StemsColor of young stem > 30 cmGreen; green with little purple pigmentation (PP).Color of young stem wings >30 cmGreen; green with little PP; green with much PP.Presence of wings on the young stemPresent; absent.Color of mature stem (1 m height)Green; green with little PP; green with much PP.Color of mature stem wings (1 m height)Green; green with little PP; green with much PP.Color of mature stem (15 cm height)Green; brownish-green; green with little PP; green with much PP; dark purple.Color of mature stem wings (15 cm height)Green; green with little PP; green with much PP.	iformity of tuber flesh color						
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Color of mature stem (15 cm height)Green; brownish-green; green with little PP; green with much PP; dark purple.Color of mature stem wings (15 cm height)Green; green with little PP; green with much PP.	e ,						
Color of mature stem wings (15 cm height) Green; green with little PP; green with much PP.							
Growing habit of the stem Straight; slightly zigzag.	lor of mature stem wings (15 cm height)	Green; green with little PP; green with much PP.					
	owing habit of the stem	Straight; slightly zigzag.					
Many lateral branches Present; absent.	iny lateral branches	Present; absent.					
Number of internodes until fully opened first leaves Five to nine; more than nine.	mber of internodes until fully opened first leaves	Five to nine; more than nine.					
Average diameter of the mature stemObtained by measuring the diameter of stem, at 15 cm height from the base of the plant, fi measurements per plant, two plants per plot in each block.	erage diameter of the mature stem	Obtained by measuring the diameter of stem, at 15 cm height from the base of the plant, five measurements per plant, two plants per plot in each block.					
Average length of the internodes Obtained by measuring the distance between leaf scars, at 1 m height from the base of plan five measurements per plant, two plants per plot in each block.	erage length of the internodes	Obtained by measuring the distance between leaf scars, at 1 m height from the base of plant, five measurements per plant, two plants per plot in each block.					
Leaves		Leaves					
Color of the mature leaf Light green; green; dark green.	lor of the mature leaf	Light green; green; dark green.					
Color of the petiole of the young leaf Green; green with slight PP at the base and junction with the leaf blade; Green with int PP at the base and junction with the leaf blade; all petiole with PP more intense at the ba junction with the leaf blade; brown; dark purple.	lor of the petiole of the young leaf	Green; green with slight PP at the base and junction with the leaf blade; Green with intensive PP at the base and junction with the leaf blade; all petiole with PP more intense at the base and junction with the leaf blade; brown; dark purple.					
Presence of wings on the petiole Present; absent.	esence of wings on the petiole						
Color of the young petiole wings Light green; green with little PP; green with many PP.	0 1						
Color of the petiole of the mature leaf Green; reen with little PP; green with much PP; green with PP at the base and junction w		Green; reen with little PP; green with much PP; green with PP at the base and junction with the leaf blade; all petiole with PP, more intense at the base and junction with the leaf blade; reddish-					

Continuation...

Continuation...

Descriptors	Characteristics
1	
Color of the mature petiole wings	Green; green with little PP; green with much PP.
Density the foliage	Low; intermediate; high.
Number of lobes	Predominantly 3; predominantly 5.
Deepness of the lobes	Less deep; very deep; very deep and less deep (two types)
Distance between the two upper lobes and the petiole	No distance; little distance; intermediate; very distant
Size of the main lobe	Particularly large (more than twice the size of other lobes); regular (same or slightly larger).
Average diameter of the leaf	Obtained by measuring the maximum diameter of the leaf area, five measurements of mature leaves per plant, two plants per plot in each block.
Average length of leaves	Obtained by measuring the maximum length of the leaf area, five measurements of mature leaves per plant, two plants per plot in each block.
Average length of petioles	Obtained by measuring the length of five petioles of mature leaves, two plants per plot in each block.
	Flowers and fruit
Months until flowering after budding	Before four months; between four and five months; between six and seven months; without flowering.
Sex	Female; male.
Number of male inflorescences	Few (50-100); medium (100-200); many (>200).
Spikes often with double flowers	Present; absent.
Spikes with lateral branches	Present; absent.
Single or double flowers in the leaf axils without spikes	Present; absent.
Number of female inflorescences	Few (1-30); medium (30-60); many (60-100).
Shape of fruit	Very oblong; less oblong, almost round.
Average number of flowers per inflorescence	Obtained by counting the flowers of five inflorescences, two plants per plot in each block.

pilosity); leaves (leaf position, border color, rib color, leaf tip color, pilosity, curl and ripple); flowers and fruit (position of the fruit, pilosity); and tubers (maturity after budding, and ability to provoke itchy skin).

The different phenotypic expressions of each descriptor facilitated the creation of an identification key for the 20 assessed landraces, as follows.

1. Leaves predominantly with three lobes.

2. Stems, petioles, and wings of the stems and petioles, without purple pigmentation; tubers with white flesh; tuber skin with very high-root density; numerous female inflorescences; flowering 1 to 2 months earlier than other landrace – "barbudo".

2. Wings of stems and petioles with purple pigmentation; light-purple tubers flesh, which are darker in the apical part (Munsell 5RP C6 V4,5,6,7); male inflorescences can form clusters with 2 to 5 flowers together in the branches of the inflorescence - "rabo-de-mucura".

1. Leaves with five lobes.

- 3. Wholly white tuber flesh.
- 4. Petioles without purple pigmentation.

5. Tuber skin with high-root density; zigzag-shaped stem growth by slightly inclined internodes; two

distinct leaf types - leaves with deep and thin lobes, and leaves with less deep and wider lobes; male inflorescences with a different shape, showing single, or double flowers in the leaf axils, without spikes – "japonês".

5. Smooth tuber skin without roots; wings of petioles without purple pigmentation; female inflorescences - "boliviano".

5. Tuber skin with few roots; wings of petioles with intense purple pigmentation; Female inflorescences – "branco-comprido".

4. Mature petioles with purple pigmentation; female inflorescences.

6. Base of the petiole is green, although the rest has a purple pigmentation; smooth tuber skin without roots; tubers with an oval shape - "ovo-de-cavalo".

6. Central part of the petiole is green, but the base and the junction with leaf blade have a purple pigmentation; tuber skin with medium-root density – "macaxeira".

3. White tuber flesh with slightly purple spots.

8. Most tubers are wider than long; female inflorescences. 9. Thin layer (≤ 1 mm) of the outermost cortex with purple pigmentation; mature petioles have

more purple pigmentation at the base, and junction with leaf blade; tubers are flat with an irregular shape - "pé-de-elefante".

8. Absent thin purple outermost cortex layer; very long petioles with purple-brown pigmentation; lobes of leaves are very deep; rounded tubers - "pé-de-burro".

8. Most tubers with a shape of equal width and length, or a bit oblong. 10. Thin layer (≤ 1 mm) of the outermost cortex with a purple pigmentation; tuber development is very distant (>1 m) from the center of the pit; tuber skin with many roots; male inflorescences - "branco-casquinha-roxa".

8. Absent thin purple outermost cortex layer; tubers relatively close to the center of the pit; female inflorescences - "branco-redondo".

3. White tuber flesh, with purple stripes, and outermost purple tuber flesh layer (0.1-0.5 cm).

9. Intensely pigmented dark-purple petioles; long and oval tubers; abundant and vigorous male inflorescences, spikes often with double flowers -"açaí-comprido".

9. Petioles with less intense purple pigmentation, more concentrated at the base, and junction with the leaf blade; wider than long tubers; less abundant and less vigorous male inflorescences - "açaí-redondo".

3. White tuber flesh in the distal part (ca. 2-3 cm layer), which are purple in the central and apical parts (Munsell 5RP C4 V3,4,5,6); petioles with purple pigmentation, but base of petioles remains green; tubers flat and irregularly shaped; male inflorescences - "pé-de-porco".

3. Wholly purple tuber flesh.

10. Dark-purple tuber flesh (Munsell 5RP V5 C4/6). 13. Large leaves with very deep lobes; tubers with thick skin, deep wrinkles, and few roots, with a slightly oblong oval shape; tubers very close to the center of the pit; nondeveloped inflorescences – "durão".

10. Main lobe of leaves much larger than the other lobes; oval and curved tubers with medium-root density; slightly oblong, almost round fruit – "preto".

3. Light-purple tuber flesh (Munsell 5RP C4/6 V6,7).

11. Tuber with a wider shape in the distal part, and flattened in the bottom; stems, petioles, and wings are very thin; young stems and petioles almost without wings; high-root density in the apical part of the tuber; male inflorescences – "casado".

11. Tubers with a wide shape and a moderate tendency to branch out to form a palm-like shape; female inflorescences - "mão-de-onça".

11. Oval and oblong-shaped tubers; male inflorescences. 15. Leaves with intermediate distance between the two upper lobes; tubers without tendency to branch - "roxo-comum".

11. Leaves with an intermediate distance, and with much distance between the two upper lobes; slight branching tendency - "roxo-de-humaitá".

The identification key represents an auxiliary tool for the identification of the landraces assessed in this study, and should be improved and complemented according to the identification and characterization of new landraces in the Amazon region.

Aside from the tuber shape, the purple pigmentation of the tuber flesh contributed notably to distinguish the landraces. There are landraces without any purple pigmentation of the tuber flesh, and others with various types of more or less intense purple pigmentation, which was also observed by Nascimento et al. (2015). In addition to the tuber flesh, the different types of the purple pigmentation of the petioles can be used as descriptors of the aerial parts, especially when no tubers are available. The purple pigmentation occurs due to anthocyanins, phenolic compounds synthesized in the secondary metabolism via the phenylpropanoid pathway. Ramos-Escudero et al. (2010) detected 12 anthocyanin compounds in D. trifida, from which the main one is the 3-O-p-coumaroylglucoside-5-Oglucoside. Tubers from one pit and one cut tuber as a sample for each assessed landrace, are shown in the Figures 2, 3, 4, and 5.

Based on the morphological data transformed into a binary matrix, the Jaccard similarity index was calculated between the pairs of landraces, then, a cluster analysis was performed and graphically displayed through a dendrogram, showing the formation of some landrace groups of great similarity, and other ones morphologically more different (Figure 6).

There are two major groups (I and II), the first one is formed by the landraces from "japonês" to "barbudo", descending the dendrogram, and the second group is formed by the landraces from "casado" to "açaí". The first group is composed of landraces with white tuber flesh, except for "rabo-de-mucura". The second group is composed of landraces that have tuber flesh with purple pigmentation.

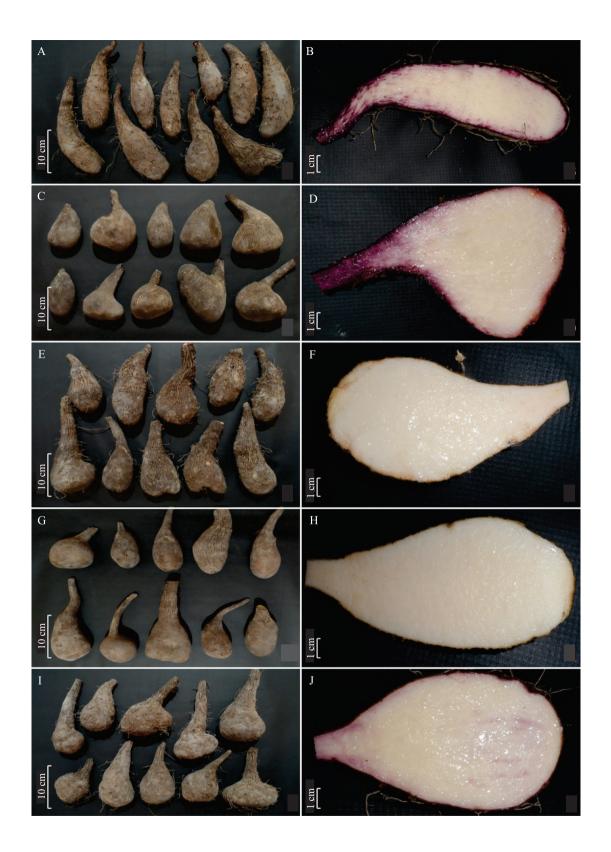


Figure 2. Tubers from one pit, and one cut tuber of each landrace of *Diocorea trifida*: "açaí-comprido" (A, B), "açaí-redondo" (C, D), "barbudo" (E, F), "boliviano" (G, H), and "branco-casquinha-roxa" (I, J).

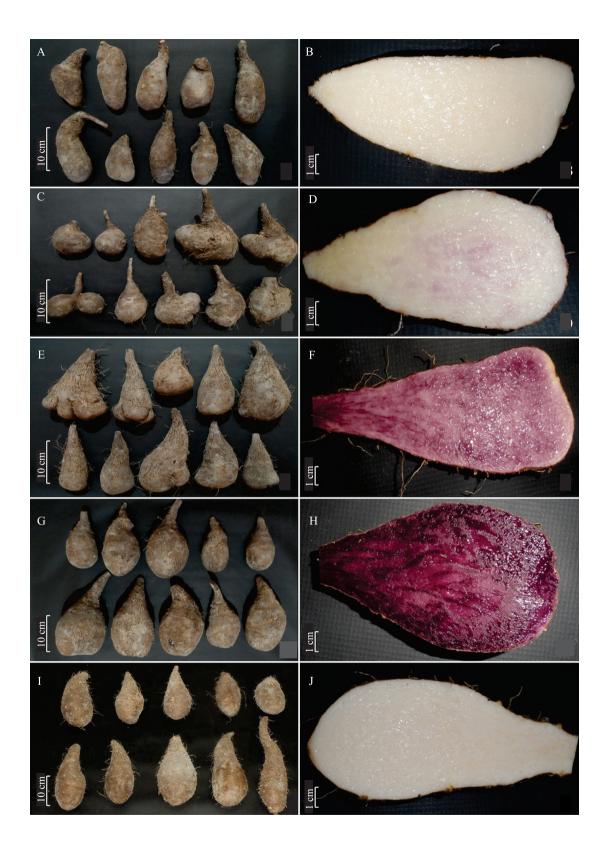


Figure 3. Tubers from one pit and one cut tuber for each landrace of *Diocorea trifida*: "branco-comprido" (A, B), "branco-redondo" (C, D), "casado" (E, F), "durão" (G, H), and "japonês" (I, J).

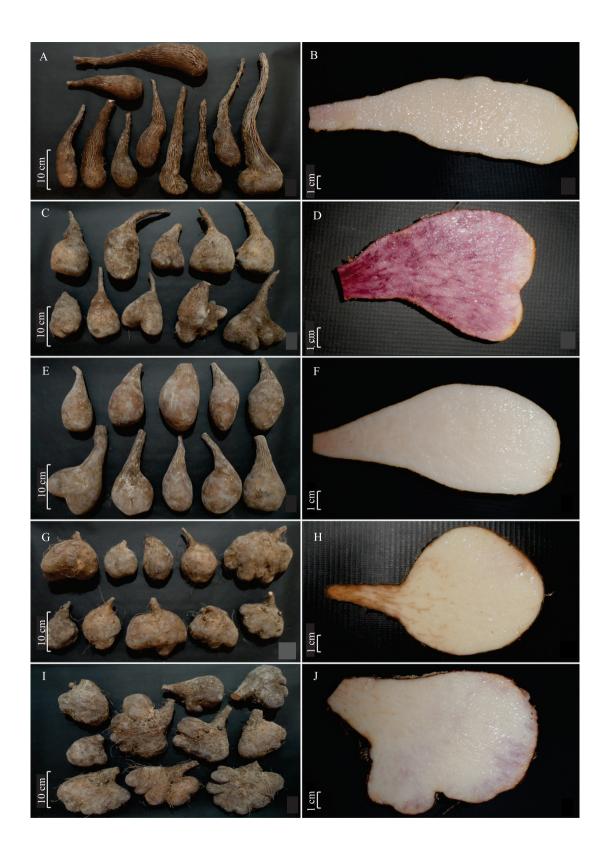


Figure 4. Tubers from one pit and one cut tuber for each landrace of *Diocorea trifida*: "macaxeira" (A, B), "mão-de-onça" (C, D), "ovo-de-cavalo" (E, F), "pé-de-burro" (G, H), and "pé-de-elefante" (I, J).

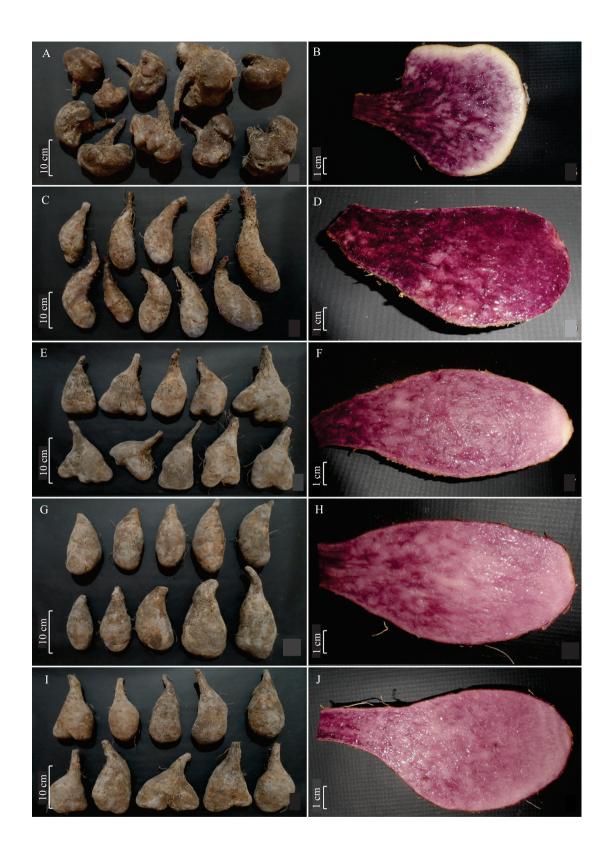
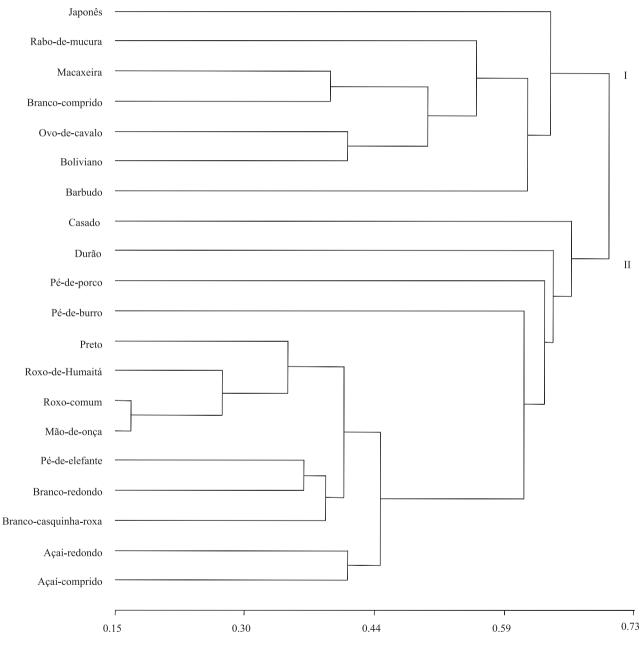


Figure 5. Tubers from one pit and one cut tuber for each landrace of *Diocorea trifida*: "pé-de-porco" (A, B), "preto" (C, D), "rabo-de-mucura" (E, F), "roxo-comum" (G, H), and "roxo-de-humaitá" (I, J).

The amplitude of the Jaccard index varied from 0.83 to 0.13, showing a considerable morphological diversity among the evaluated landraces. The pair of landraces with the highest Jaccard index was "roxo-comum" and "mão-de-onça", indicating that these landraces have the highest morphological similarities, and show the smallest distance 0.17 (1-S).

In the group I, the landrace "japonês" is the most morphologically distinct. This landrace is cultivated in commercial crops by Japanese descendants of the metropolitan area of Manaus, and may have resulted from an intensive domestication process. The second most distinct landrace of the first group is "barbudo", which is cultivated by a farmer in the community Lago



Distance: Jaccard (1-S)

Figure 6. Cluster analysis based on 49 morphological descriptors of 20 landraces of *Dioscorea trifida*, using the Jaccard index and the UPGMA cluster method.

de Arara, in the municipality of Caapiranga. According to the farmer, this landrace appeared in the cultivation area spontaneously, without being planted. If this is correct, the landrace "barbudo" is possibly the result of sexual reproduction. The landrace "rabo-de-mucura" is within group I, among the landraces with white tuber flesh, probably because this landrace with purple pulp has relatively little purple pigmentation in the aerial parts of the plant. As registered in many other regions (Bardsley, 2006), Caapiranga small farmers not only act to conserve local agrobiodiversity, but they also strive to increase it by incorporating new materials.

Another landrace that could be the result of a sexual reproduction in the cultivation area is "casado", which, according to the farmers, is a result of a "marriage" between a purple and a white yam. "casado" is in group II, with the highest morphological distance. The landrace with the second highest distance within group II is "durão", that has peculiar morphological characteristics, such as very large leaves, large tubers with thick skin and deep wrinkles.

The landrace "roxo-de-humaitá", a commercial variety from the municipality of Humaitá, at 592 km distant from Manaus, is morphologically close to "roxo-comum", the main commercial landrace of Caapiranga. The other landraces that come from distant municipalities - from Boa Vista do Ramos, a municipality at 269 km from Manaus -, are "pé-deporco" and "preto", that also do not show a particular morphological distance relatively to the other landraces. This may indicate that genetic exchange between the distant regions has probably occurred. This is consistent with the results of Nascimento et al. (2013), who observed only a low correlation between the genetic and geographical distances, which indicates the anthropogenic interference in the distribution of genetic variability of the species.

Conclusions

1. A set of 48 morphological descriptors is required to properly and efficiently identify a group of 20 landraces of *Dioscorea trifida*.

2. The identification key for the landraces of *D*. *trifida* provide a tool to facilitate the in situ management of the genetic resources, and it should serve as a basis for a participatory breeding program to rescue and recognize the value of this native food crop.

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