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Chromosomal analysis of eight cultivars in three species of cultivated Yam (*Dioscorea* L.) species in Nigeria

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Abstract. The genus *Dioscorea* comprises of economically-important plant species known for their starch throughout the world; it is also a major source of food and income in Africa. The most important *Dioscorea* species cultivated and consumed in the West Africa belt include *D. cayenensis*, *D. rotundata* and *D. alata.* The plant materials used in this study were collected from Omu-Ekiti, Oye Local Government of Ekiti State, Nigeria using the On Farm Participatory Method (OFPM). Mitotic chromosome studies were carried out on three species viz, *Dioscorea alata* ('ewura'), *D. cayenensis* ('igangan') and *D. rotundata* ('areyingbakumo', 'gaungaun', 'ikumo', 'ogunmole' and 'sandpaper'). Mitotic chromosome studies were carried out on each of the cultivars using the root tip squash method made in modified Orcein stain (FLP-Orcein). Dormant tubers were cut to mini-setts and placed in carbonised rice husk for rooting. This study reports the basic chromosome number of x = 8, i.e. 2n = 4x =32 (*D. alata*), 2n = 4x =38 (*D. rotundata*) and 2n = 8x = 68 (*D. cayenensis*) for *Dioscorea* suggesting that both *D. rotundata* and *D. cayenensis* are aneuploids.

Keywords: Dioscorea, Chromosomes, aneuploids, polyploids, mixoploidy.

INTRODUCTION

The genus *Dioscorea* L. belongs to the family Dioscoreaceae which includes about 90% of the species in the family (Murti 2001). The genus *Dioscorea* is principally tuber-bearing and has great economic value in the tropics as food, pharmaceutical, starch, socio-cultural uses and source of income to farmers in West and Central Africa (Asiedu et al. 1998; Séka et al. 2009). The most important *Dioscorea* species cultivated and consumed in the West Africa belt include *D. cayenensis* Lam., *D. rotundata* Poir. and *D. alata* L. (IITA 2009). West African countries produce over 90% of world *Dioscorea*, of which, Nigeria is the largest producer of *Dioscorea* in the world, producing over 60% of the world *Dioscorea* (FAO 2016; 2017).

Dioscorea has presented a challenge to systematists for many years due to its great morphological diversity, its reproductive biology: dioecy, small flowers, low or no seed set and tuber propagation (Wilkin et al. 2005). Nor-

man et al. (2012) has reported the difficulty of chromosome studies in yams (*Dioscorea* spp.) due to the small dot-like chromosomes and few dividing cells in the root tips. Also, mixoploidy has been reported to be characteristic of many highly productive commercial cultivars with small chromosome sizes (Kunakh 2005; Kunakh et al. 2008). Mixoploidy was reported by Baquar (1980) in some *Dioscorea* species studied which he tagged "odd chromosome numbers"

Dioscorea alata was reported to have the highest diversity in polyploidy ranging between 2n = 30 and 2n = 80 (Sharma and Deepesh 1956; Franklin and Oritz 1963; Egesi et al. 2012). The ploidy levels of *D. cayenensis* have been reported to range between a tetraploid (2n = 4x = 40) and an octoploid (2n = 8x = 80) on a basic chromosome number of 10 (Baquer 1980; Gamiette et al. 1999; Dansi et al. 2001). A basic chromosome number of 20 has been suggested using *D. cayenensis* (Dora et al. 2005) and *D. alata* (Arnau et al. 2009).

Flow cytometry has offered some advantages in ploidy level analysis (Babil et al. 2010). Two ploidy levels (4x and 8x) were detected by flow cytometry in two populations of *D. cayenensis* and *D. rotundata* cultivars (Dansi et al. 2000; Babil et al., 2010). However, this technique has failed to detect aneuploidy in this population. Therefore, Babil et al. (2010) recommended the use of classical chromosome studies to determine ploidy levels and solve the complication of mixoploidy in the genus *Dioscorea*. Babil et al. (2010) then recommended that determination of the basic chromosome number of *Dioscorea* spp. requires further investigations. Norman et al. (2012) advocated the need for chromosome studies which is necessary to clarify the structure, function, organisation and evolution of yam genomes.

The aim of this study was to investigate the chromosome number of the cultivars in the three major *Dioscorea* species that are present in Nigeria using the squash technique. The results presented will be useful both in the identification and understanding of the phylogenetic relationship among the major cultivars in the three major species of *Dioscorea*.

METHODOLOGY

The plant materials used in this study were collected from Omu-Ekiti (N 07.90497' E 005.39092') in the Oye Local Government of Ekiti State, Nigeria. This community typifies an epicentre of loss of genetic resources as a result of mass adoption of introduced *Dioscorea* cultivars by migrant farmers from the Middle Belt of Nigeria in the last twelve years. Mitotic chromosome studies were carried out on seven cultivars in three species: *Dioscorea alata*, *D. cayenensis* and *D. rotundata*. Dormant tubers were cut into mini-setts placed in carbonised rice husk for rooting. The root tips were harvested between 10.30–11.30 am, rinsed in water and transferred into 1:3 Acetic Ethanol fixative. This was left on the working bench for 24 h at room temperature before keeping in the refrigerator for future usage. The root tips for examination were hydrolysed in 18% HCl for 10 min, squashed and stained with FLP-Orcein. Photomicrographs of the good mitotic chromosome spreads were documented under oil immersion (x1000) objective magnification using BK Series Phase Contrast Microscope (PW-BK 5000T) equipped with a DCM510 5 Megapixel camera. The chromosome numbers were based on five consistent counts.

RESULTS

Phenotypic variation in leaf characters

Table 1 and Figure 1 show the forms and shapes of the leaves of some of the *Dioscorea* species studied. All the cultivars studied had simple, glabrous, cordate leaves. The petiole of *D. alata* ('ewura') had purple wing which was absent in other species. The leaves of *D. rotundata* ('areyingbakumo') and *D. cayenensis* ('igangan') cultivars had orbiculate (broad cordate) leaves.

Table 1. Leaf characteristics of the Yam cultivars studied.

Species	Local name	Foliar description
Dioscorea alata	Ewura	Green colour, ovate shape, acuminate apex, cordate base and winged petiole.
Dioscorea cayenensis	Igangan	Light green colour, orbicular shape, acuminate apex and cordate base.
Dioscorea rotundata	Areyingbakumo	Green colour, orbicular shape, acuminate apex and cordate base.
	Gaungaun	Green colour, ovate shape, acuminate apex and sagittate base.
	Ikumo	Green colour, orbicular shape, acuminate apex and cordate base.
	Ogunmole	Green colour, ovate shape, acuminate apex and cordate base.
	Sandpaper	Dark green colour, long ovate shape, acuminate apex and sagittate base.

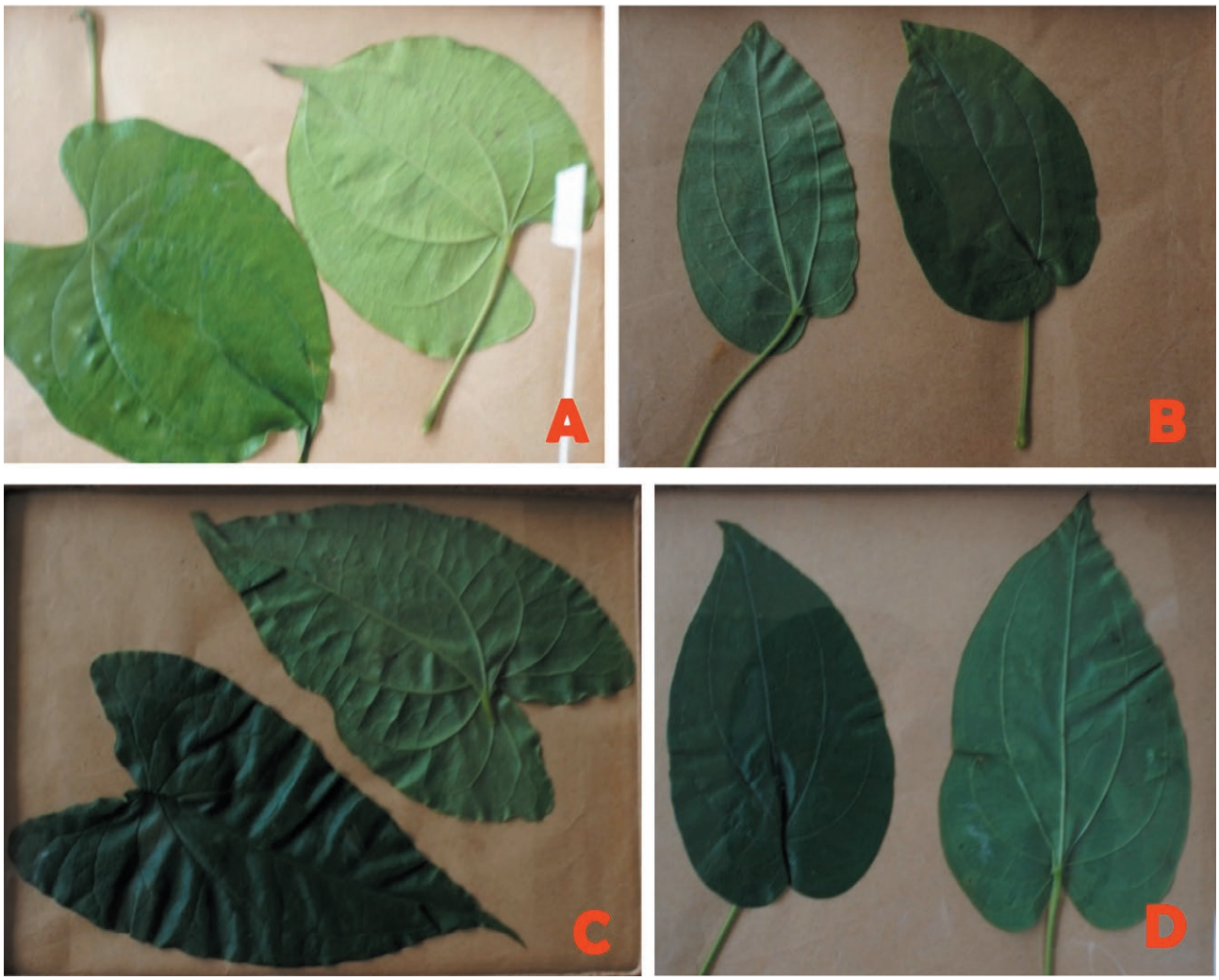

Figure 1. Leaf form and shape in some the cultivars of *Dioscorea* studied. **(a)** *D. cayenensis* **(b)** *D. rotundata* **(c)** *D. rotundata* **(d)** *D. rotundata*

However, the leaf colour of *D. cayenensis* was light green while that of *D. rotundata* were dark green. Only, *D. rotundata* ('sandpaper') had long cordate leaves. The cultivars had acuminate leaf apices. The leaf bases observed in *D. rotundata,* ('gaungaun' and 'sandpaper' cultivars) were sagittate while other *D. rotundata* cultivars studied had cordate leaf bases.

Chromosome number and morphology

The mitotic chromosome counts observed in the *Dioscorea* species studied were shown in Figures 2 and 3. A mitotic chromosome count of 2n = 32 was observed in *D. alata* cultivar while a mitotic chromosome count of 2n = 68 was observed in the *D. cayenensis* cultivar (Figure 2). The mitotic chromosome count observed in all the *D. rotundata* cultivars was 2n = 38 (Figures 2 and 3). Table 2 shows the mitotic chromosome counts in this study and the previous chromosome counts. The morphology of the chromosomes could not be ascertained in this study because of their small sizes.

DISCUSSION

The findings of this study revealed morphological variations between and within the leaves of the cultivars of the *Dioscorea* species studied. As established in Table 1 and Figure 1, the distinct by its winged petiole of *D. alata* distinguishes it from delimit the *D. rotundata* and *D. cayenensis* cultivars while can be. Also, leaf colour

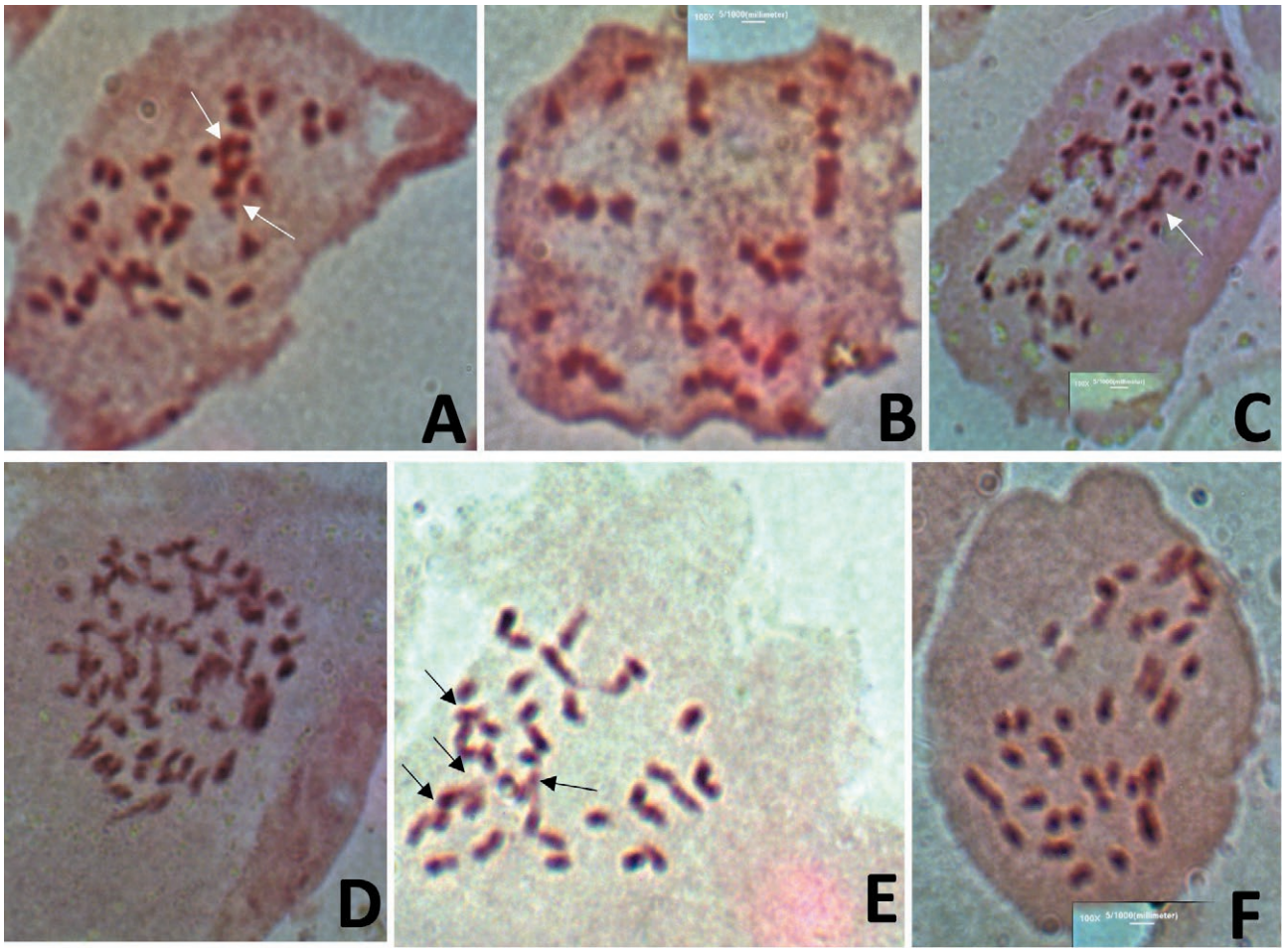

Figure 2. Mitotic metaphase spreads in the *Dioscorea* species studied. A. *D. alata* (Ewura), 2n = 32 (Arrows show mitotic chromosome overlaps); B. *D. alata* (Ewura), 2n = 32; C. *D. cayenensis* (Igangan), 2n = 68 (Arrow shows mitotic chromosome overlap); D. *D. cayenensis* (Igangan), 2n = 68; E. *D. rotundata* (Ikumo), 2n = 38 (Arrows show mitotic chromosome overlap); F. *D. rotundata* (Ikumo), 2n = 38.

serves as a delimiting character, *D. cayenensis* has light-green leaves while *D. rotundata* has dark-green leaves and *D. alata* leaves are green. The orbicular leaf shape of *D. cayenensis* distinguish it from some *D. rotundata* and *D. alata* cultivars. However, in the morphological characterisation of *Dioscorea*, there is possibility of overlap of characters, therefore, the use of multiple delimiting features is important for their characterisation.

This study established a chromosome number of 2n = 38 for the four cultivars of *Dioscorea rotundata,* 2n = 32 for *D. alata* and 2n = 68 for *D. cayenensis.* None of the mitotic counts observed in this study is in agreement with the previous mitotic chromosome numbers reported for yam (Table 1). (Bousalem et al. 2006) reported that the dot-like and varying chromosome sizes that occurred in the mitotic cells of *Dioscorea* made the definite determination of chromosome numbers difficult. The mitotic chromosome counts reported in this study were smaller compared to the mitotic chromosome numbers earlier reported. Asiedu et al. (1998) had reported the occurrence of smaller chromosome numbers and polyploidy levels in the species of *Dioscorea* from Asia and Africa.

This study reports the basic chromosome number of x = 8 in *D. alata* (2n = 4x =32), *D. rotundata,* (2n = 4x =38) and *D. cayenensis* (2n = 8x = 68). The x = 8 basic chromosome number agrees with the findings of (Dansi et al. 2001). There was no mixoploidy observed. Baquar (1980) reported odd chromosome numbers that were not direct multiples of their basic chromosome numbers which he tagged "odd chromosome numbers". The study cytogenetics of *Dioscorea* using roots from tubers could give a better result in terms chromosome morphology and stainability compared to vines generated roots.

The findings indicate that *D. rotundata* (2n = 38) and *D. cayenensis* (2n = 68) are distinct species. This

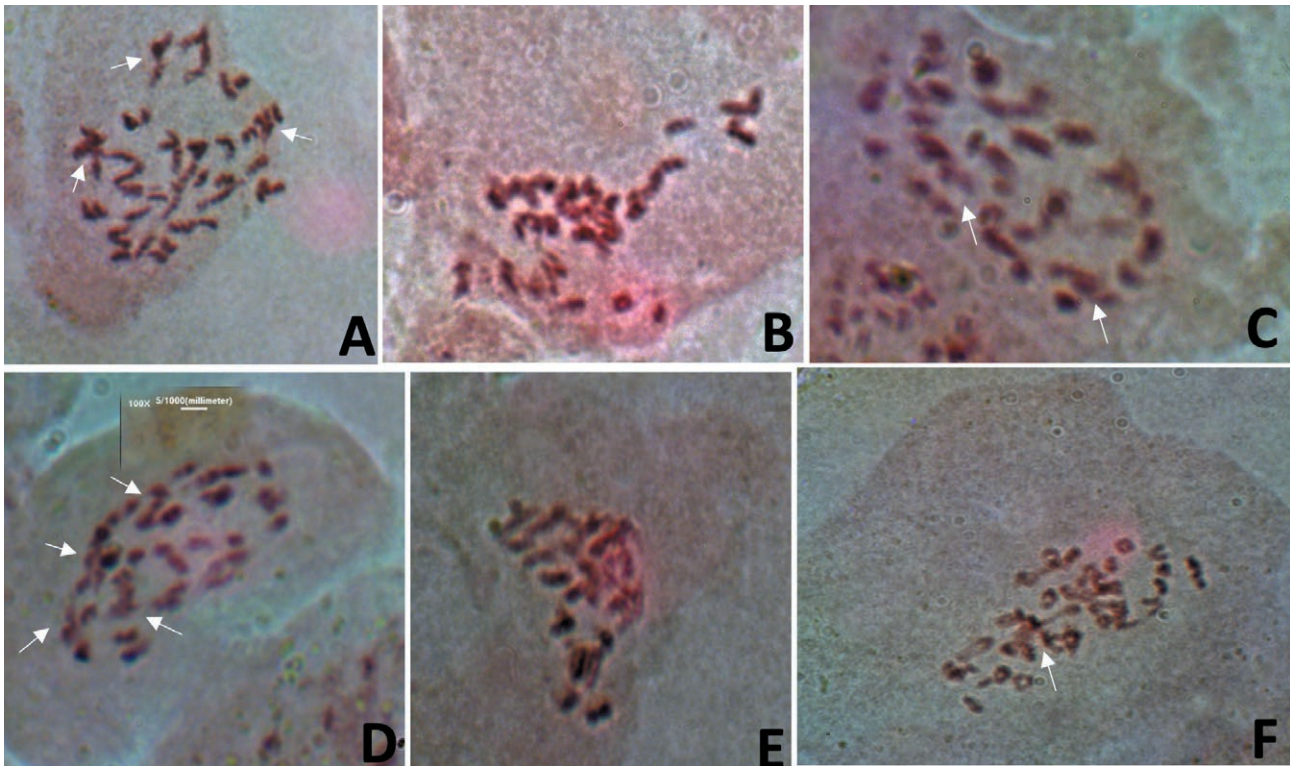

Figure 3. Mitotic metaphase spread in *Dioscorea* species studied. A. *D. rotundata* (Gaungaun), 2n = 38 (Arrows show chromosome overlaps); B. *D. rotundata* (Gaungaun), 2n = 38; C. *D. rotundata* (Sandpaper), 2n = 38 (Arrows show chromosome overlaps); D. *D. rotundata* (Ogunmole), 2n = 38 (Arrows show chromosome overlaps); E. *D. rotundata* (Areyingbakumo), 2n = 38; *D. rotundata* (Areyingbakumo), 2n = 38 (Arrow shows chromosome overlap)

study affirms that *D. cayenensis* is a distinct species from *D. rotundata* thus corroborating the results of Bressan et al. (2014) who classified the two species separately through isozymatic analysis. *D. cayenensis* might be a speciated polyploid of *D. rotundata* based on the leaf morphology and the chromosome count reported in this study. *Dioscorea* is principally propagated vegetatively, hence, *D. cayenensis* could have arose through the process somatic cell divisions and polyploidy. The probability that this occurrence could have been as a result of abnormality in the somatic cell divisions of the planting materials (Sharma and Deepesh 1956; Stebbins 1971; Baquar 1980) is considered remote.

Polyploidy has been reported in domesticated plants which include *Dioscorea* species (Lewis 1980; Leitch and Leitch 2008; Jeredi et al. 2012). Based on a basic number of x = 8 (Dansi et al., 2001), *D. alata* (2n = 4x =32) is a tetraploid and *D. rotundata* (2n = 38) can only be an aneuploid trisomic for six linkage groups while *D. cayenensis* (2n = 68) would be an octaploid with four trisomic sets.

CONCLUSION

The chromosome numbers reported in this work are based on five consistent counts for all the cultivars. It is difficult to agree that mixoploidy is an issue in the chromosome numbers of the cultivars studied because the analysable cells did not show wide variations in chromosome number. On the other hand, for a crop that is maintained by clonal propagation, the occurrence of multiple chromosome numbers is not impossible, especially since a cultivar is not a taxonomic hierarchy. Rather, it is characterized by a cluster of valuable food and agronomic attributes that have distinguished it for selection and conservation through generations of cultivation by peasant farmers.

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Table 2. Present and previous reports of chromosome numbers in the genus *Dioscorea*.

Species	Present	Previous	Reference
D. alata	32	20	Sharma and Sharma (1957)
		30	Sharma and Deepesh (1956)56
		40	Miége (1954); Simmond (1954); Sharma and Deepesh (1956), Raghavan (1958), Martin and Ortiz (1963); Ramachandran (1962; 1968); Baquar (1980)
		50	Sharma and De (1956); Baquar (1980)
		60	Raghavan (1948); Martin and Ortiz (1963); Ramachandran (1962; 1968).
		70	Sharma and De (1956)
		80	Raghavan (1948); Ramachandran (1962; 1968); Baquar (1980)
		81±	Nakajima (1936).
D. cayenensis	68	34	Miége (1954).
		36	Miége (1954).
		54	Miége (1954).
		60, 62, 63, 66±	Miége (1954); Baquar (1980)
		80	Baquar (1980)
		140	Smith, 1937
D. rotundata	38	40	Martin and Ortiz (1963); Baquar (1980)
		60	Baquar (1980)

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