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KARYOLOGICAL AND ANATOMICAL STUDIES ON *SYNGONIUM PODOPHYLLUM* SCHOTT (ARACEAE)

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ABSTRACT

Anatomical and cytological studies were carried out on Syngonium podophyllum which belongs to the family Araceae. Anatomical studies were achieved using free-hand sectioning and the leaves were peeled with the aid of forceps. The species is amphistomatic having isotrycytic, tetracytic and anomocytic stomata. The anatomy of the stem, petiole, leaf lamina and leaf midrib revealed the presence of scattered bicollateral and leptocentric vascular bundles; calcium oxalate crystals (druses) and starch grains which are widely distributed in different parts of the plant. The druses help to protect the plant against insect and animal infestation. It has chromosome number of 2n = 24 (n = 12). The length of the chromosomes ranged from 2.43 ± 0.10 μm to 4.95 ± 0.07 μm and the karyotype has 7-metacentric chromosomes and 5-submetacentric chromosomes (with karyotype formula = 7m+5sm) which is reported for the first time in Nigerian species.

INTRODUCTION

Syngonium podophyllum Schott belongs to Araceae family with more than 105 genera and 3300 species (Mayo et al., 1997) distributed in the tropical and temperate regions of the world (Croat, 1981). S. podophyllum as an invasive weed of banana plantations (Garita, 2003) can grow fast forming dense colonies that cover native vegetation (Possley, 2004; ISSG, 2012; PIER 2012) and displace native species by changing plant community structures (Florida Exotic Pest Plant Council, 2011; ISSG, 2012). This species can spread through seeds and stem cuttings (Croat, 1981; Space and Flynn, 2000; ISSG, 2012) and could be dispersed by birds and mammals (Chong et al., 2010). However, this species has some economic importance ranging from medicinal purposes (Croat, 1994), ornamental values (Sipos and Trif, 2009) and eliminating polluting agents from inside the households (Wolverton et al., 1984),

Several researches have been carried out on S. podophyllum some of which include morphological (Rav, 1992), anatomical studies including type and location of the calcium oxalate crystals in the leaves (Genua and Hillson, 1985); variety and distribution of the oxalate crystals (Franceschi and Horner, 1980); physiological studies, in vitro and ex vitro propagation (Salame and Zieslin, 1994), somatic embryogenesis of the species (Graur and Adholeya 1999; Zhang et al., 2006;); genetic analysis (Chen et al., 2006) and leaf protein analysis (Wafaa et al., 2010). Among the Araceae family, calcium oxalate crystals synthesized within specialized cells have been reported in at least 215 species. These calcium oxalates have different morphological characteristics and are classified based on their forms as druses (crystal aggregates), raphides (long pointed needles bundled within cells), prisms and very fine crystal sand (Keating, 2003, 2004). These crystals act as defense mechanism for the plant against insect or animal infestation (Gardner, 1994; Uno et al., 2001; Barabé et al., 2004; Osuji, 2013)

Araceae family is known to have wide variation in chromosome numbers of 2n = 14 to 140 and in chromosome size (Marchant, 1973), a primary basic number of x = 6, 7, or 8, (Mabberley, 1997); x = 8 to 22 or mostly 14 (Jones, 1957). Chromosomal data have played an important role in the study of plant systematics and evolution at all levels (Mabberley, 1997). It has been used to solve many problems in the delimitation of various plant taxa (Moore, 1978). In the genus Syngonium, chromosome number 2n = 22, 24, 26

and 28 have been reported (Marchant, 1971; Guha and Bhattacharya, 1987; Subramanian and Munian, 1988; Petersen, 1989) while chromosome number of x = 12, 2n = 24 (Guha and Bhattacharya, 1987), 2n = 26 (Petersen, 1989) and 2n = 22 (Mohamed et al., 2006) have been reported in S. podophyllum. Despite these works on S. podophyllum and other aroids, there has been relatively little study on karyotype of S. podophyllum. Therefore we investigated the anatomy, mitotic index and karyotype of S. podophyllum from Nigeria.

MATERIALS AND METHODS

Source of Material

The plant materials used in this study were collected from the University of Port Harcourt Biodiversity Center and properly identified and deposited in the Department of Plant Science and Biotechnology Herbarium.

Petiole, Midrib and Stem studies

Petiole, stem and midrib portions of S. podophyllum were fixed in formalin, acetic acid and alcohol (FAA) (in ratio of 1:1:18 parts of 70% ethanol v/v) for 12 h. Thereafter, the specimens were dehydrated in ethanol of different concentrations (30 and 50%) and stored in 70% ethanol until when needed. The leaf portion of the petiole, stem and the central portion of the leaf midrib were sectioned following the method of Agbagwa et al. (2007). The sections were stained in Alcian blue and counter-stained with 1% Safranin O for two minutes, mounted on a slide, viewed and photographed with an Optika B-1000 FL LED microscope.

Epidermal Studies

Foliar materials for epidermal studies were collected fresh from plants. The adaxial and abaxial epidermal peels were obtained using sharp pointed forceps. Peels were stained with 1% Safranin O or Alcian blue, rinsed with distilled water to remove excess stain and mounted in a drop of pure glycerine on clean glass slides. A cover slip was placed over the drop and sealed with nail vanish to prevent dehydration (Okoli and Ndukwu, 1992). The epidermal features that were observed include: organization of the epidermis, arrangement of the epidermal cells, shape of epidermal cells and nature of the anticlinal cell wall of the leaf epidermis, stomatal types and index. The stomatal index (SI) was determined according to Metcalfe and Chalk (1979), while the terminology for the stomatal type description is according to Malvey (2004).

Cytological Studies

Root tips of about 1cm long were excised and pretreated in 0.002 M 8-hydroxyquinoline for 3–3.5 h, Fixed in 3:1 ethanol -acetic acid for 24 h and the roots were stored in 70% ethanol solution before squashing. When required the roots were removed from the 70% ethanol, hydrolyzed in 10% HCl, squashed in a drop of FLP orcein (Osuji, 2003). Mitotic chromosomes were observed under Optika B-1000 FL LED research microscope and photomicrographs of five good quality metaphase plates were taken and recorded. The long arm (l), short arm (s) and the total chromosome length (c) of each chromosome were measured on enlarged microphotographs. The relative lengths, arm ratios (r = l/s) and centromeric index (I = s/c 100) were calculated and used to classify and determine homologous chromosomes (Gomurgen et al. 2010). For karyotype description the chromosomes were arranged in groups according to the chromosome length and in order of decreasing size in each class. Chromosome nomenclature followed Levan et al. (1964). The variation in chromosome length and chromosome arm ratio within the karyotype was estimated by calculating mean and standard deviation (SD) of these parameters using Microsoft Excel 2010.

RESULTS AND DISCUSSION

Epidermal Characteristics

S. podophyllum is amphistomatic. Tetracytic, anomocytic and isotricytic stomata types were found on the abaxial surface while only tetracytic stomata was found on the adaxial surface (Figure 1a and b). Tetracytic stomata were the dominant stomata and the stomatal indices are 1.33 ± 1.34 and 11.31 ± 0.75 on adaxial and abaxial surfaces respectively. The abaxial and adaxial epidermal cells are pentagonal to heptagonal in shape and partly polygonal or rectangular with mainly curved and partly straight anticlinal cell walls. Osuji and

Nwala (2015) identified stomata with similar morphological features in Xanthosoma (L.) Schott and Colocasia (L.) Schott (Araceae) however, they called them paracytic stomata. The presence of anomocytic stomata type has been reported among Araceae family (Sipos and Trif, 2009; Ekeke and Agbagwa, 2016). In addition, Ekeke and Agbagwa (2016) reported isotricytic and anisocytic stomata in Caladium bicolor and noted that this species is amphistomatic with isotricytic and anisocytic stomata occurring only on the abaxial leaf surface while Sipos and Trif (2009) reported tetracytic and hexacytic (anomocytic) stomata in S. podohpyllum. The result of the epidermal characteristics of S. podophyllum is in agreement with the findings of

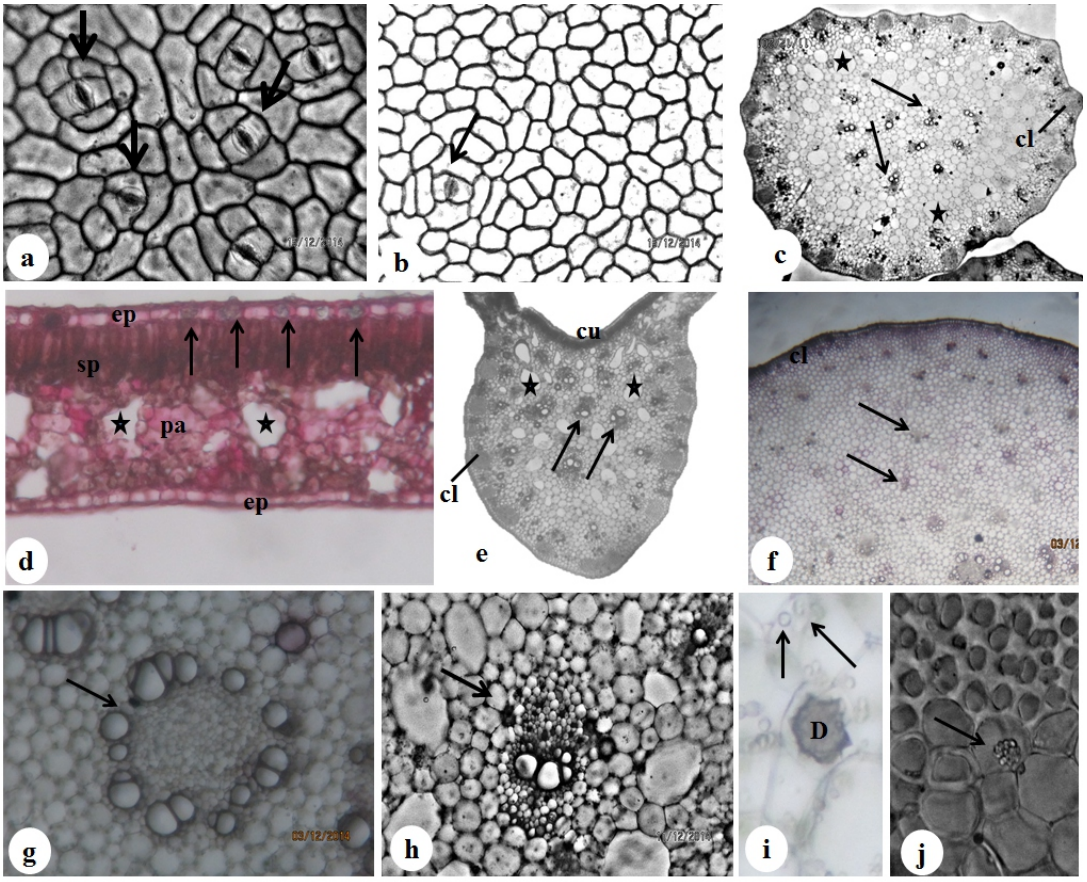

Figure 1: Epidermal and Anatomical Characteristics of S. podophyllum **(×100)**

a – adaxial epidermis (arrows show stomata); b – abaxial epiderimis (arrow shows stoma); c – petiole (arrows show vascular bundles, stars show intercellular air spaces); d – leaf lamina (arrows show calcium oxalate crystals, stars show intercellular air spaces); e – midrib (arrows show vascular bundles, stars show intercellular air spaces); f – stem (arrows show vascular bundles); g – arrow shows concentric (leptocentric) vascular bundles; h – arrow shows bicollateral vascular bundles; i – plant tissues (arrows show starch grains and D shows calcium oxalate crystal-druse) and j – plant tissue (arrow shows cluster of crystals), cl = collenchyma, ep = epidermis, pa = palisade mesophyll, sp = spongy mesophyll, cu = cuticle.

Anatomical Characteristics

The petiole is irregular in shape with 21 patches or non-continuous collenchyma cells at the periphery, 10-vascular (bicollateral) bundles in the ground tissues and intercellular air spaces (Figure 1c) with undulating or wavy outer cuticle. The leaf lamina comprised periclinally elongated upper and lower epidermis, single layer of palisade mesophyll but rarely 2-layers, armed spongy cells with 6-8 layers and calcium oxalate crystals (druses) in the upper epidermis and spongy mesophyll (Figure 1d).

The outline of the midrib section is V-shaped and has non-continuous peripheral collenchyma (21 in number), 15 concentric (leptocentric) or bicollateral vascular bundles and druses embedded in the ground tissues with intercellular spaces (Figure 1e). The stem comprised numerous vascular bundles (leptocentric and bicollateral) scattered all over the parenchymatous ground tissues with continuous layer of collenchyma cells (Figure 1f). In the species studied leptocentric vascular bundles are predominantly found in the stem (Figure 1g) and bicollateral vascular bundles are widely distributed (Figure 1h) while druse crystals (Figures 1i and 1j) and starch grains (Figure 1i) were observed in different tissues of the plant.

In a similar study, Cardoso et al. (2013) reported starch grains in the ground meristem of Garcina brasiliensis Mart. (Clusiaceae). In S. podophyllum, many authors have reported intercellular spaces, starch grains, leptocentric and collateral closed bundles (Sipos and Trif, 2009), druses in the sub-epidermal mesophyll and rarely in the - palisade mesophyll tissues and intercellular spaces in mesophyll (Saadi and Mondal, 2012) and raphide-containing cell (biforine) (Fochtman et al., 1969). Gary (2009) and Ekeke and Agbagwa (2016) noted wide distribution of druses in some Araceae. Their findings are similar to our observations. For instance we found druses in almost all plant parts but in the lamina they are restricted to the upper epidermal cells and the palisade mesophyll (Figure 1d) but in contrast with the occurrence of druses in palisade and spongy mesophylls in Caladium bicolor (Ekeke and Agbagwa, 2016). These calcium oxalate crystals form part of the plant defense mechanism (Uno et al., 2001; Osuji, 2013) due to the presence of toxic proteins (Fochtman et al., 1969), glucosides (Saha and Hussain, 1983), or other toxins (Ladeira et al., 1975). The crystals could then function to irritate, scratch or stab toxins into the herbivore's tissues (Saadi and Mondal, 2012).

Cytological characteristics

The mitotic study showed that cell division in S. podophyllum is regular and the different stages of cell division are shown in figure 2 while the peak of metaphase is between 12:00 noon and 4:00 pm. This suggests that the best time to collect root samples for metaphase study is few minutes between 1:00 pm and 3:00 pm (Figure 3). Ekanem and Osuji (2006) also suggested that the best time to harvest roots for metaphase in Xanthosoma and Colocasia species is immediately before 12:00

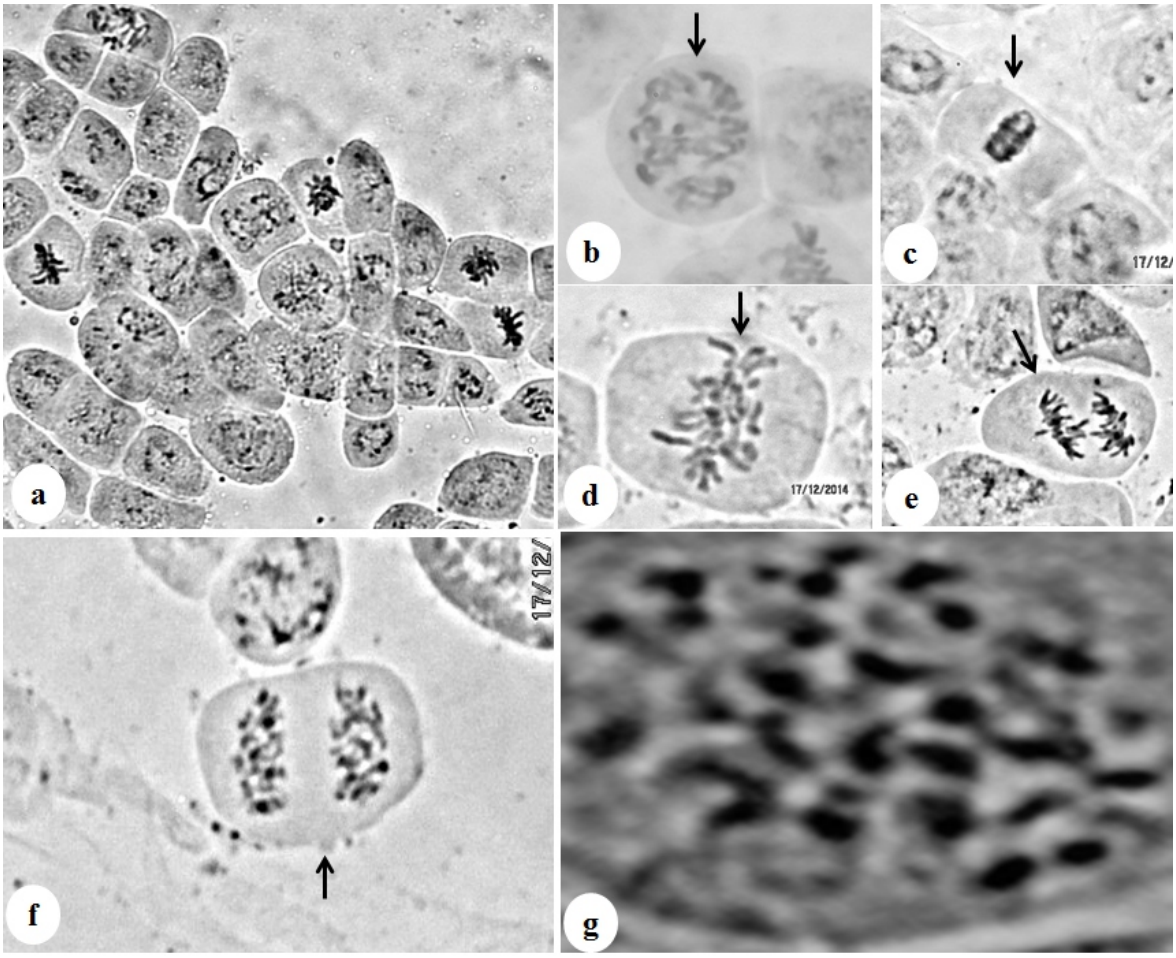

Figure 2: **Mitotic Cell Division in** S. podophyllum
a – cells at different stages of division x200; b – interphase x400; c – prophase x400; d – metaphase x400; e – anaphase x400; f – telophase x500 and g – mitotic chromosome (2n = 24) x800.

Mitotic chromosome count of 2n = 24 suggesting n = 12 was recorded in S. podophyllum (Figure 2g) and the karyotype idiogram is shown in figure 4. The karyotype comprised seven metacentric chromosomes and five submetacentric chromosomes (Table 1) suggesting karyotype formula of 7m+5sm. The length of the chromosome pairs varied from 2.43 ± 0.01 μm to 4.95 ± 0.07 μm, the chromosome long arms are 1.44 ± 0.08 μm to 3.13 ± 0.18 μm and short arms - 0.99 ± 0.10 to 1.83 ± 0.11 μm (Table 1). The arm ratio ranged from 1.23 to 2.12. Chromosomes 4 and 7 had satellites. Previous works have recorded chromosome number of 2n = 24 (Guha and Bhattacharya, 1987), 26 (Marchant, 1971; Petersen, 1989), 22 (Subramanian and Munian, 1988; Mohamed et al., 2006) and 28 (Petersen, 1989) in S. podophyllum. The finding of this work is in contrast with the findings of Guha and Bhattacharya (1987) and further reported karyotype formula of 7m+5sm for the first time.

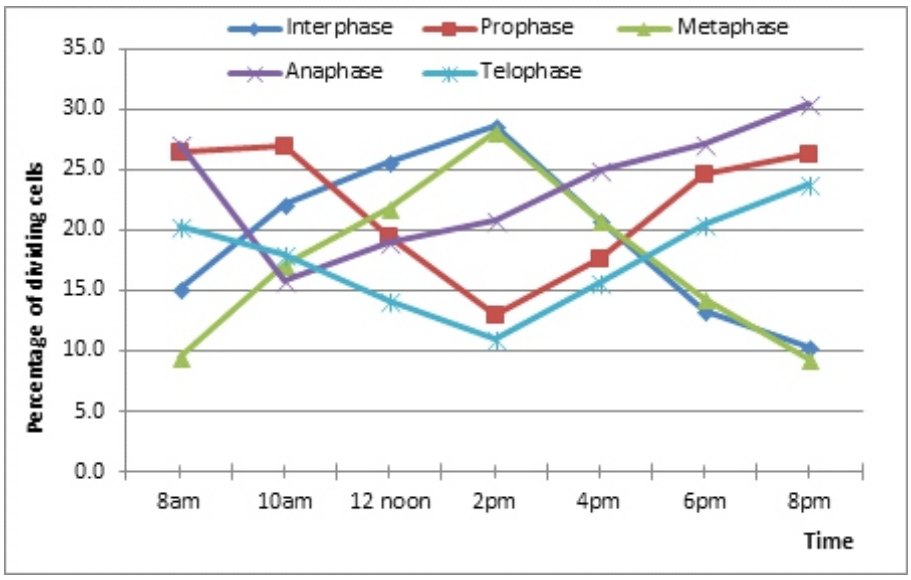

Figure 3: Dynamics of Mitosis Showing Relative Percentage of Cells at the Different Stages of Mitosis at Different Hours of the Day in S. podophyllum

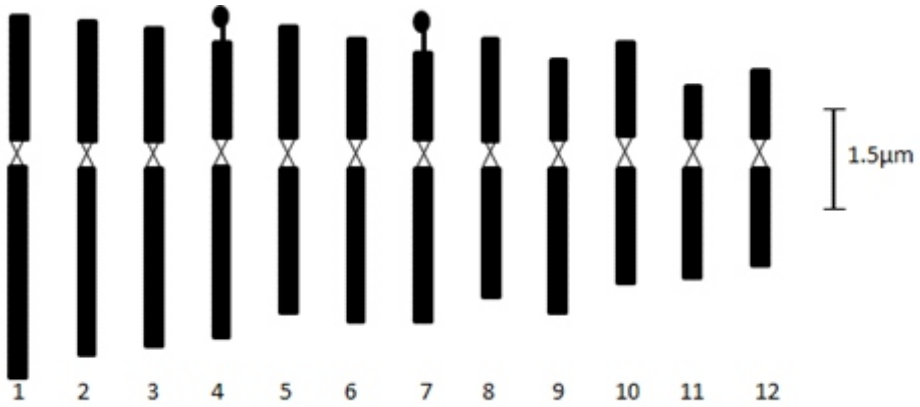

Figure 4: **Haploid Idiogram of** S. podophyllum

Table 1: **Karyotype Data on Arm Lengths and Centromic Positions** of Syngonium podophyllum

Chromosome pair	Long arm (μm) l	Short arm (μm) s	Total length (μm)	Arm ratio (r=l/s)	I	Centromeric position
1	3.13 ± 0.18	1.83 ± 0.11	4.95 ± 0.07	1.71	36.97	sm
2	2.75 ± 0.35	1.78 ± 0.04	4.53 ± 0.39	1.54	39.34	m
3	2.63 ±0.18	1.66 ± 0.13	4.28 ± 0.31	1.58	38.79	m
4	2.52 ± 0.02	1.43 ± 0.11	3.94 ± 0.13	1.76	36.29	sm
5	2.13 ± 0.18	1.65 ± 0.14	3.78 ± 0.04	1.29	43.71	m
6	2.24 ± 0.02	1.45 ± 0.07	3.69 ± 0.09	1.54	39.35	m
7	2.25 ± 0.01	1.28 ± 0.04	3.52 ± 0.03	1.76	36.36	sm
8	1.90 ± 0.15	1.53 ± 0.18	3.42 ± 0.03	1.24	44.74	m
9	2.13 ±0.18	1.18 ± 0.11	3.30 ± 0.07	1.81	35.76	sm
10	1.69 ± 0.27	1.37 ± 0.19	3.06 ± 0.08	1.23	44.84	m
11	1.63 ± 0.18	0.77 ± 0.02	2.47 ± 0.05	2.12	31.24	sm
12	1.44 ± 0.08	0.99 ± 0.10	2.43 ± 0.10	1.45	40.74	m

Note: Mean±SD, I = Centromic index (Ratio of short arm to the total chromosome length × 100), m = Metacentric and sm = Submetacentric.

CONCLUSION

S. podophyllum has starch grains and druse crystals widely distributed in different parts (stem, petiole, leaf lamina and midrib) of the plant. The druses probably help to protect the plant against insect and animal infestation. The chromosome number is 2n = 24 (n = 12). The length of the chromosomes ranged from 2.43 ± 0.10 μm to 4.95 ± 0.07 μm and the karyotype has 7-metacentric chromosomes and 5-submetacentric chromosomes (with karyotype formula = 7m+5sm) which is reported for the first time in Nigerian species.

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