KARYOTYPE STUDIES IN SOME SPECIES OF THE FAMILY ANNONACEAE.

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Abstract

Seeds of various accessions of *Annona muricata, Greenwayodendron suaveolens* and *Cleistopholis patens* were collected from the wet forest, dry forest and derived savanna areas of Nigeria to determine their karyotype. Each of the species has a chromosome number of n = 7 (2n = 14). A pair of satellited chromosomes was observed in the genome of *Greenwayodendron suaveolens* and *Cleistopholis patens*. The centromeric positions in the species of Annonaceae investigated are nearly median with predominance of large chromosome types. The karyotypes were similar within the species studied, with small intra-and inter-generic variations in terms of size. The karyotypic patterns suggest that there is no evidence of chromosome rearrangement in their evolution.

Key words: Karyotype, satellited, chromosome, rearrangement, evolution

1. Introduction

The Annonaceae belongs to the class Magnolideae in the order Magnoliales. Cronquist (1968), Mabberley (1987) and Brummitt (1992) reported that the family consists of 2,050 species in 125 genera and they are found mainly in the tropics. Many members of this family are of great economic importance, most especially the species studied in this work. The fruits and leaves of *Annona muricata* are used in traditional medicine in tropical America and West Indies because of their tranquillizing and sedative properties (Hasrat, De Bruyne, De Backer, Vauquelin and Vlietinck, 1997).

The seeds of *Annona muricata* contain a yellow oil which is applied to hair in India and Mexico to kill lice. *Greenwayodendron suaveolens* is used for landscaping, its leaf extract was evaluated against the larvae and pupae of the mosquito species *Culex quinque fasciatus*. It significantly inhibited the emergence of adults (Murty, Sriram and Kaiser, 1997). Folorunso and Olorode (2002, 2006) have reported the crude protein distribution in *Annona muricata, Greenwayodendron suaveolens* and *Cleistopholis patens*.

Studies of chromosome variation in Annonaceae have shown a rich array of chromosomal variations in many groups (Morawetz, 1984; 1984a; 1984b; 1986; 1986a). In this family, twenty six genera are diploid with 2n = 26 while thirty other genera have base numbers of x = 7, x = 8, or x = 9. Others show polyploidy, associated with ecological specialization.

The constancy and usefulness of the karyotype arises from the fact that, at a given stage of cell division and in a given tissue, each cell of an organism has a constant number of chromosomes of reasonably definite volume, length and shape (Adedeji and Faluyi, 2003).

Up to date, no karyotype data are available for the species of Annonaceae in Nigeria. This work presents a comparative study of the karyotype of *A. muricata, Greenwayodendron suaveolens* and *Cleistopholis patens*. It is hoped that this will stimulate further studies in the family Annonaceae.

2. Materials and Methods

Living plant materials were collected from different ecological zones such as the wet forest, dry forest and derived savanna areas of Nigeria (Table 1). Seeds of various accessions were germinated in plastic buckets and transplanted directly into the soil in the experimental garden in Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. Herbarium materials were prepared from all the collections and deposited as voucher specimens in the herbarium of Obafemi Awolowo University, Ile-Ife.

Mitotic studies were carried out using root tip squashes made in modified orcein stain (FLP – Orcein) described by Olorode (1974). Root tips from seedlings grown in plastic buckets were collected between the hours of 9.00 a.m. and 11.00 a.m. when mitotic activities are believed to be usually high

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Table 1: Accession studied and the location of collection

Species	Accession Number	Location
Annona muricata	1	Botanical Garden, O.A.U., Ile-Ife. Osun State 7° 30" N 4° 31" E, Nigeria.
"	2	Behind Biochemistry Dept. O.A.U., Ile-Ife. Osun State 7° 30" N 4° 31" E, Nigeria.
	3	Botanical Garden, U.I., Ibadan, Oyo State 7° 23" N 3° 56" E, Nigeria.
	4	Within Ilesa Town, Osun State 7° 37" N 4° 44" E, Nigeria.
	5	Along Road 7,Ile-Ife, Osun State 7° 30" N 4° 31" E, Nigeria.
	6	Within Ilorin Town, Kwara State 8° 32" N 4° 34" E, Nigeria.
	7	Along Ibadan- Ife Road,Oyo State 7° 30" N 4° 31" E, Nigeria.
"	8	Behind Botany Dept. O.A.U.,Ile-Ife, Osun State 7° 30" N 4° 31" E, Nigeria.
"	9	Along Ondo-Ore Road, Ondo State 7° 15" N 5° 14" E, Nigeria.
"	10.	Along Eti-Oni Road, Atakumosa 7° 38" N 4° 75" E, Nigeria.
Greenwayodendron suaveolens	1	Along Road 7,Ile-Ife, Osun State 7° 30" N 4° 31" E, Nigeria.
"	2	Along Ibadan- Ife Road,Oyo State 7° 30" N 4° 31" E, Nigeria.
"	3	Botanical Garden, U.I., Ibadan, Oyo State 7° 30" N 4° 31" E, Nigeria.
"	4	Within Ilesa Town, Osun State 7° 37" N 4° 44" E, Nigeria.
"	5	Within Ilorin Town, Kwara State 8° 32" N 4° 34" E, Nigeria.
"	6	Along Eti-Oni Road, Atakumosa 7° 38" N 4° 75" E, Nigeria.
"	7	Along Ondo-Ore Road, Ondo 7° 15" N 5° 14" E, Nigeria.
"	8	Along Akure Road, Ilesa 7° 37" N 4° 44" E, Nigeria.
"	9	Along Road 20, O.A.U.,Ile-Ife. Osun State 7° 30" N 4° 31" E, Nigeria.
"	10	Within Uniben. Campus, Benin 7° 37" N 4° 44" E, Nigeria.
Cleistopholis patens	1	Teaching and Research Farm, O.A.U., Ile-Ife. Osun State 7° 30" N 4° 31" E, Nigeria
"	2	Along Ede Road, Ile-Ife. Osun State 7° 30" N 4° 31" E, Nigeria
"	3	Within Ilesa Town, Osun State 7° 37" N 4° 44" E, Nigeria
"	4	Along Iperindo Road, Ilesa 7° 37" N 4° 44" E, Nigeria.
"	5	Within Ilorin Town, Kwara State 8° 32" N 4° 34" E, Nigeria.
"	6	Along Eti-Oni Road, Atakumosa 7° 38" N 4° 75" E, Nigeria.
	7	Along Ondo-Ore Road, Ondo 7° 15" N 5° 14" E, Nigeria.
	8	Along Ondo-Benin Road, Ondo 7° 15" N 5° 14" E, Nigeria.
	9	Within Akure Town, Akure. 7° 15" N 5° 14" E, Nigeria.
	10	Along Road 20, O.A.U.,Ile-Ife. Osun State 7° 30" N 4° 31" E, Nigeria.

(Jackson, 1962). The harvested root tips were then pretreated in 8-hydroxyquinoline solution for two to three hours before fixing in 1:3 acetic acid ethanol fixative. The fixed root tips were left at room temperature for at least 24 hours and used immediately thereafter or stored in a refrigerator until used later. Slides were prepared by the squash method after softening the roots for 5-10 minutes in 18% HCl and washed in distilled water. A flat monolayer of cells was ensured by gentle tapping on the cover slip with the base of a ball point pen and then applying a gentle thumb pressure on the cover slip. Good cells were photographed under the oil immersion phase contrast objective of Leitz Dialux research microscope.

Chromosome measurements were made in millimetres from photomicrographs of somatic cells which were enlarged 2,580 times. The chromosomes were matched or paired by a physical analysis of the chromosomes based on arm length and arm ratio measurement data based on the methods described by Torres (1968) and Qurecky (1970). The chromosomes in the karyogram were numbered and arranged in order of decreasing length. The long arm to short arm ratios (L/S), centromeric index (C.I.), and shortest/longest chromosomes length ratios were calculated for the species genomes.

3. Results and Discussion

Plates 1A-C show the mitotic metaphase chromosomes of *Annona muricata*, *Greenwayodendron suaveolens* and *Cleistopholis patens* respectively. Each of these species has a chromosome number of 2n = 14 on a basic number of 7. A pair of satellited chromosomes was observed in the genome of *Greenwayodendron suaveolens* and *Cleistopholis patens* (Plates 1B and 1C).

Plate 2(A-C) and Fig. 1-3 show the karyotypes and idiograms of *Annona muricata*, *Greenwayodendron suaveolens* and *Cleistopholis patens*, respectively while Table 2-4 show the basic chromosomes parameters. All the chromosomes of the species of Annonaceae investigated were nearly median. In *Annona muricata*, chromosome pairs 1, 2 and 4 were long; the remaining chromosomes were medium in size. In both *Greenwayodendron suaveolens* and *Cleistopholis patens*, chromosomes 1-5 are long, while chromosomes 6 and 7 are medium in size. In *Annona muricata* the longest chromosome (chromosome 1) had the length of 2.5 ± 0.38 mm and the shortest chromosome measured 1.43 ± 0.25 µm (chromosome 7). In *Greenwayodendron suaveolens* the longest chromosome had the length of 3.22 ± 0.13 µm while the shortest chromosome

Table 2: Karyotypic data of *Annona muricata*

Chromosome Pair Number	S (mean ± s.d.) μm	L (mean ± s.d.) μm	S+L (mean ± s.d.) μm	TCL%	Arm Ratio (L/S)	C.I.	C.P.	C.T.
1	0.98 ± 0.13	1.52 ± 0.13	2.50 ± 0.38	18.5	1.55	39.29	nm	A
2	0.80 ± 0.12	1.25 ± 0.24	2.06 ± 0.32	15.2	1.56	39.13	nm	A
3	0.80 ± 0.12	1.16 ± 0.12	1.97 ± 0.25	14.6	1.44	40.91	nm	B
4	0.71 ± 0.25	1.34 ± 0.13	2.06 ± 0.45	15.2	1.87	34.78	nm	A
5	0.80 ± 0.12	1.16 ± 0.12	1.97 ± 0.25	14.6	1.44	40.91	nm	B
6	0.71 ± 0.25	0.80 ± 0.12	1.52 ± 0.06	11.3	1.13	50.00	nm	B
7	0.53 ± 0.00	0.89 ± 0.00	1.43 ± 0.25	10.6	1.66	37.50	nm	B
7	5.33	8.12	13.51	100.00				
(mean ± s.d.)	0.76 ± 0.14	1.16 ± 0.25	1.93 ± 0.36	14.28 ± 2.45	1.52 ± 0.21	40.36 ± 4.40		

N.B.: S = Short arm length (mean ± s.d.)
L = Long arm length (mean ± s.d.)
S+L = Total chromosome length (mean ± s.d.)
TCL% = Total chromosome length percentage = (S + L/∑(S+L)) X 100
C.P. = Centromere position: m = median, nm = nearly median, nsm = nearly submedian
C.I. = Centrometric index = (S/S+L) X 100
C.T. = Chromosome type: A, B or C
A greater than or equal to 2.01 μm = long
B = 1.01-2.0 μm = medium

Table 3: Karyotypic data of *Greenwayodendron suaveolens*

Chromosome Pair Number	S(mean ± s.d.) μm	L(mean ± s.d.) μm	S+L(mean ± s.d.) μm	TCL%	Arm Ratio (L/S)	C.I.	CP	C.T.
1	1.52 ± 0.13	1.70 ± 0.13	3.22 ± 0.13	19.15	1.11	47.22	nm	A
2	1.16 ± 0.12	1.70 ± 0.13	2.86 ± 0.57	17.00	1.47	40.00	nm	A
3	0.98 ± 0.12	1.43 ± 0.25	2.41 ± 0.31	14.30	1.46	40.63	nm	A
4	0.89 ± 0.25	1.34 ± 0.13	2.23 ± 0.31	13.25	1.51	39.90	nm	A
5	1.07 ± 0.00	1.16 ± 0.13	2.24 ± 0.06	13.30	1.08	48.00	nm	A
6	0.89 ± 0.12	1.07 ± 0.25	1.97 ± 0.13	11.70	1.20	45.20	nm	B
7	0.80 ± 0.71	1.07 ± 0.0	1.88 ± 0.19	11.17	1.33	42.60	nm	B
3	7.31	9.43	16.82	100.00				
(mean ± s.d.)	1.01 ± 0.24	1.35 ± 0.27	2.40 ± 0.44	14.36±2.85	1.47 ± 0.29	42.55 ± 4.78		

N.B.: S = Short arm length (mean ± s.d.)
L = Long arm length (mean ± s.d.)
S+L = Total chromosome length (mean ± s.d.)
TCL% = Total chromosome length percentage = (S + L/∑(S+L)) X 100
C.P = Centromere position: m = median, nm = nearly median, nsm = nearly submedian
C.I. = Centrometric index = (S/S+L) X
C.T. = Chromosome type: A,B or C
A greater than or equal to 2.01 μm = long
B = 1.01-2.0 μm = medium
C greater than or equal to 1.0 μm

Table 4: Karyotypic data of *Cleistopholis patens*

Chromosome Pair Number	S(mean ± s.d.) μm	L(mean ± s.d.) μm	S+L(mean ± s.d.) μm	TCL%	Arm Ratio (L/S)	C.I.	C.P.	C.T.
1	1.61 ± 0.25	2.06 ± 0.13	3.67 ± 0.31	21.03	1.28	43.90	nm	A
2	1.25 ± 0.25	1.70 ± 0.13	2.95 ± 0.31	16.92	1.36	42.42	nm	A
3	1.16 ± 0.13	1.79 ± 0.00	2.95 ± 0.45	16.92	1.54	39.39	nm	A
4	0.89 ± 0.00	1.43 ± 0.25	2.32 ± 0.38	13.33	1.60	38.46	nm	A
5	0.98 ± 0.12	1.16 ± 0.13	2.15 ± 0.13	12.31	1.18	45.83	nm	A
6	0.80 ± 0.13	0.89 ± 0.25	1.70 ± 0.06	9.74	0.93	47.37	nm	B
7	0.80 ± 0.13	0.89 ± 0.0	1.70 ± 0.06	9.74	0.93	47.67	nm	B
3	7.49	9.92	17.44	99.99				
(mean ± s.d.)	0.92 ± 0.44	1.40 ± 0.44	2.49 ± 0.73	14.28 ± 3.88	1.26 ± 0.25	43.53 ± 3.36		

N.B.: S = Short arm length (mean ± s.d.)
L = Long arm length (mean ± s.d.)
S+L = Total chromosome length (mean ± s.d.)
TCL% = Total chromosome length percentage = (S + L/∑(S+L)) X 100
C.P = Centromere position: m = median, nm = nearly median, nsm = nearly submedian
C.I. = Centrometric index = (S/S+L) X 100
C.T. = Chromosome type: A,B or C
A greater than or equal to 2.01μm = long
B = 1.01-2.0μm = medium

was 1.88 ± 0.19 μm in length. In *Cleistopholis patens* the longest chromosome measured 3.67 ± 0.31 μm while the shortest chromosome had a length of 1.70 ± 0.06 μm.

The three species studied for chromosomal analysis have a chromosome number of (n = 7) 2n = 14 (Morawetz 1984, 1984a and 1986). Karyotype analysis showed that chromosome size varied from 3.67 ± 0.31 μm in *Cleistopholis patens* to 1.43 ± 0.25 μm in *Annona muricata*. The centromeric positions of the species of Annonaceae investigated are nearly median with predominance of large chromosome types.

The karyotypes were fairly similar within each species, with small intra- and inter-generic variations in terms of size. There is little variability among the chromosomes of the species. The symmetry of the karyotypes suggests that chromosome rearrangement is probably not involved in the evolution of the species.

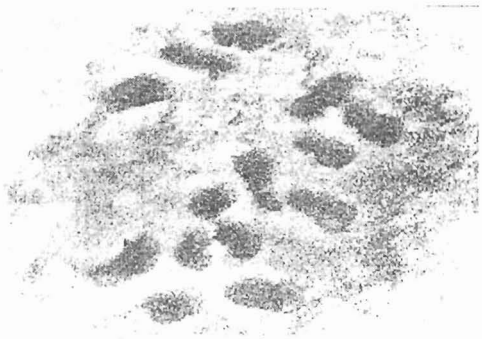

Plate 1A

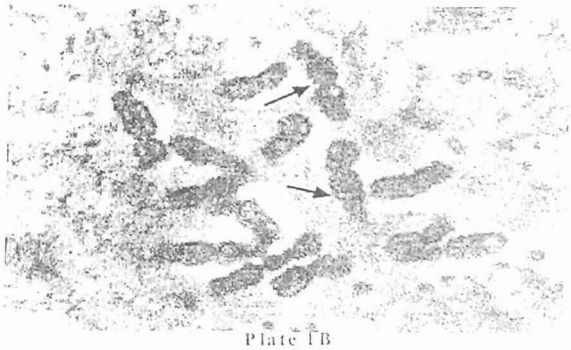

Plate 1B

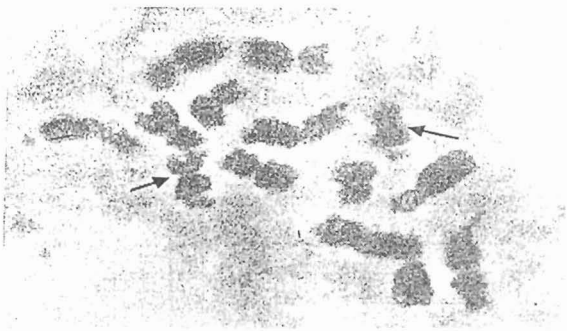

Plate 1C

6 µm

Plate 1A: Mitotic metaphase chromosome of *Annona muricata*

Plate 1B: Mitotic metaphase chromosome of *Greenwayodendron suaveolens*
Arrows indicate satellited chromosome pair.

Plate 1C: Mitotic metaphase chromosome of *Cleistopholis patens*
Arrows indicate satellited chromosome pair.

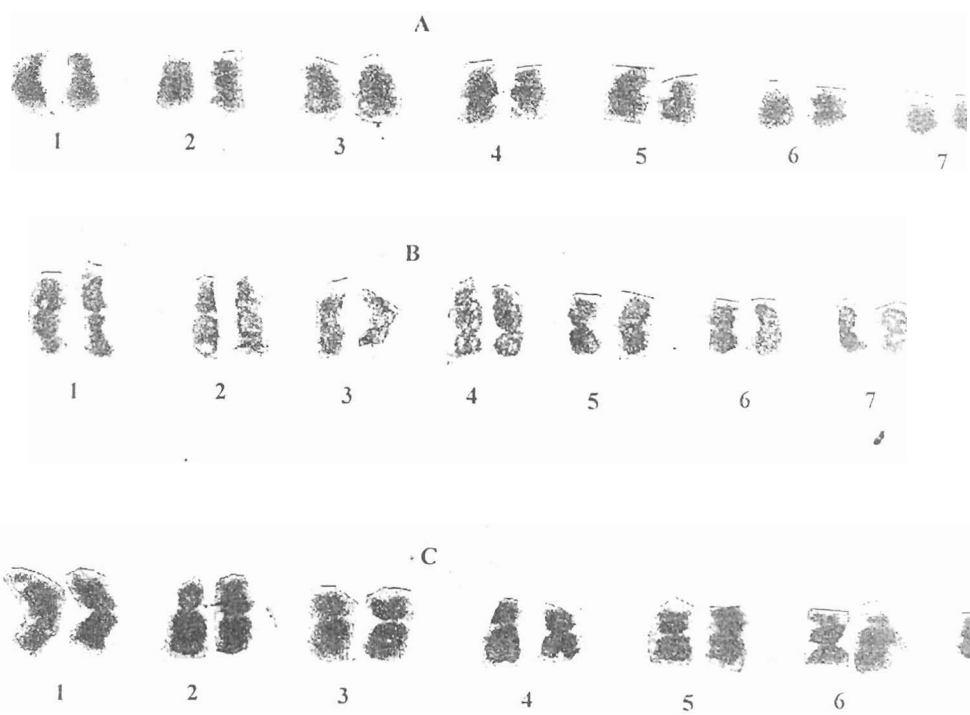

Plate 2: Karyotypes of some species of Annonaceae
A = *Annona muricata*
B = *Greenwayodendron suaveolens*
C = *Cleistopholis patens*
Chromosome 4 in B and 6 in C are satellited chromosomes

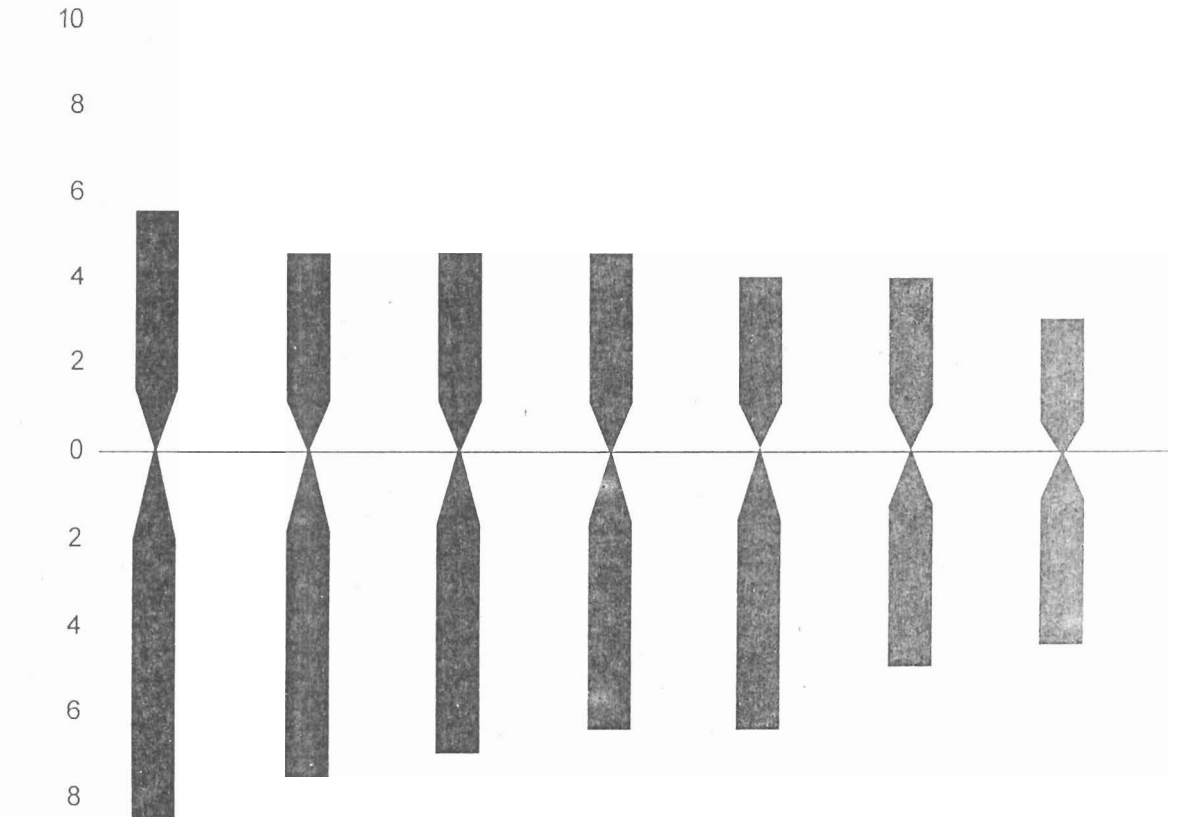

Fig. 1: Idiogram showing the haploid chromosome complement of the *Annona muricata* studied. n = 7

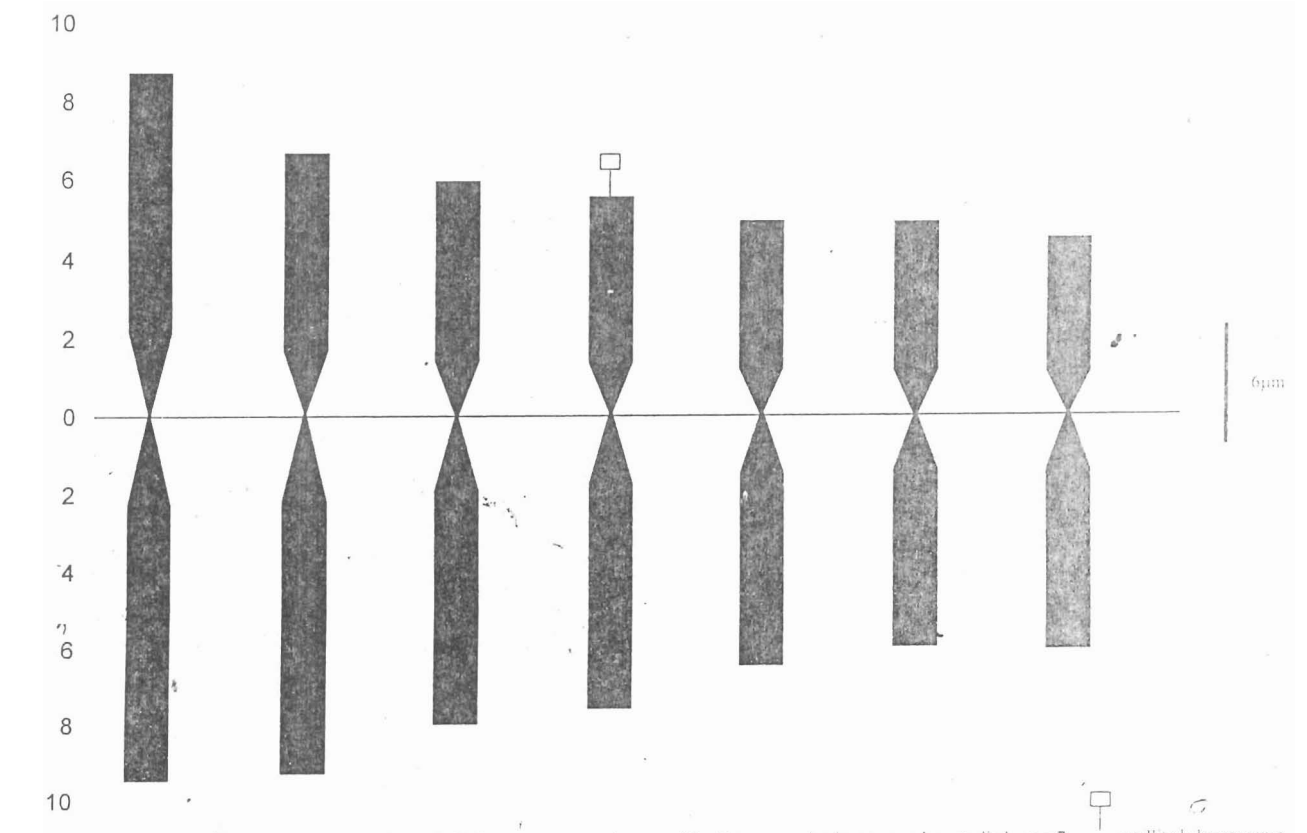

Fig. 2: Idiogram showing the haploid chromosome complement of the *Greenwayodendron suaveolens* studied. n = 7. satellited chromosome.

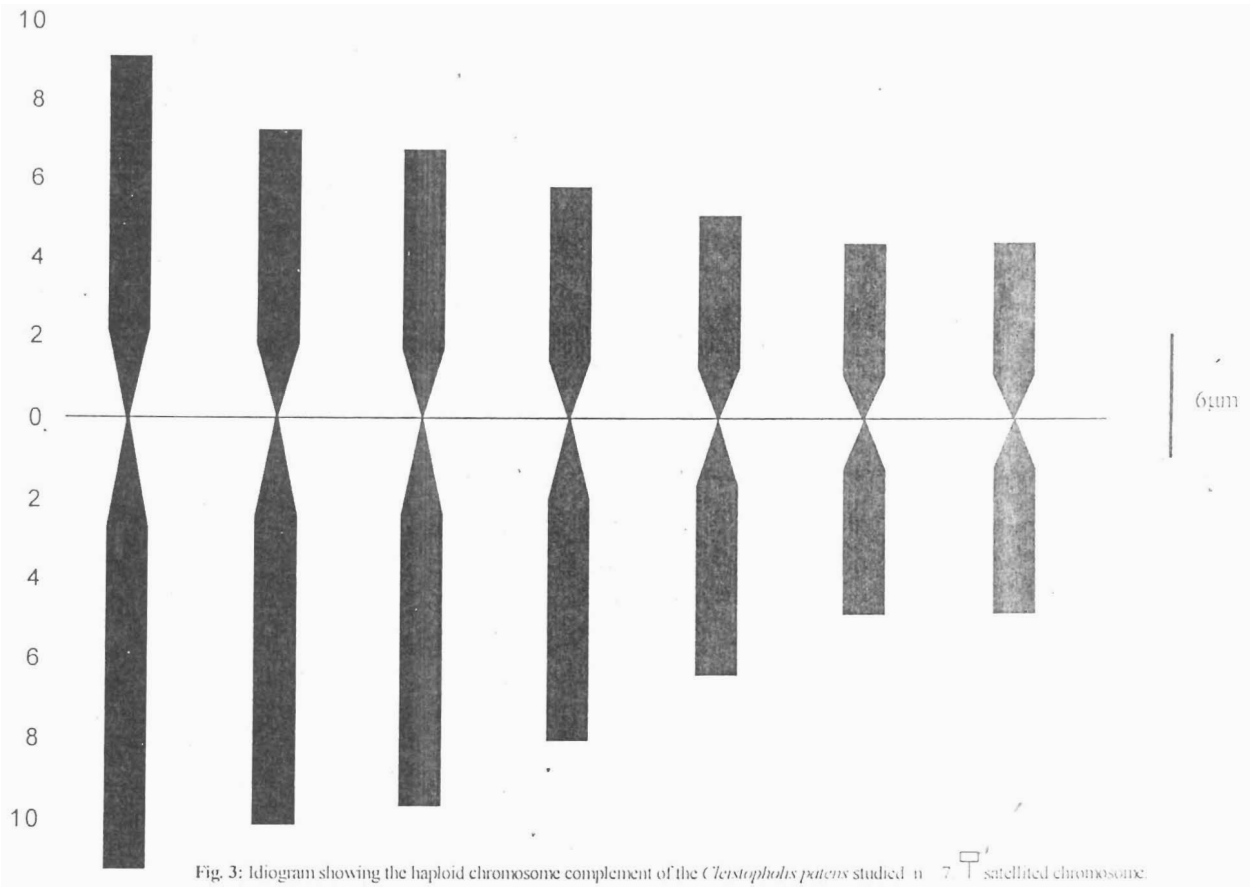

Fig. 3: Idiogram showing the haploid chromosome complement of the *Clerstopholis patens* studied n = 7, ' satellited chromosome.

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