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Chromosomal studies of species of Rubiaceae (A. L. de Jussieu) from the Brazilian cerrado

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Abstract — The chromosomes of 14 species belonging to two subfamilies of the family Rubiaceae collected in the Brazilian cerrado were studied. In the subfamily Ixoroideae, five species (*Alibertia concolor*, *A. edulis*, *A. sessilis*, *Genipa americana* and *Tocoyena formosa*) had a chromosome number of 2n = 22 and one (*Amaioua intermedia*) showed polyploidy, with 2n = 66. All of the chromosome numbers were multiples of the more common basic number for this family (x = 11). In the subfamily Rubioideae, variations in the number of chromosomes were observed among tribes and genera, with 2n = 20 in the tribe Coccocypseleae (*Coccocypselum lanceolatum*), 2n = 28 in Spermacoceae (*Borreria latifolia*, *B. verticillata* and *Richardia brasiliensis*) and n = 11 in Coussareeae (*Coussarea hydrangeifolia*). In the tribe Psychotrieae, the variations included n = 11 (*Palicourea rigida*), 2n = 22 (*Psychotria hoffmannseggiana*) and 2n = 32 (*Psychotria deflexa*). The ideograms elaborated for 12 species were symmetrical (TF% varied from 47.45 to 38.01). The size of the chromosomes ranged from 1.26 to 7.33 µm and most were metacentric. The results show that chromosome size and morphology were insufficient to differentiate among groups within the Rubiaceae.

Key words: Chromosome numbers, cytotaxonomy, Ixoroideae, karyotypes, Rubiaceae, Rubioideae.

INTRODUCTION

The family Rubiaceae, which includes about 637 genera and approximately 10,700 species, is the fourth largest of the angiosperms (ROBBRECHT 1988). This family consists of highly diversified habits, including herbs, shrubs, trees and even lianas. Most genera are cosmopolitan, with greater diversity in tropical and subtropical regions (JUDD *et al.* 1999). The rich diversity of the Rubiaceae has been confirmed in various surveys of Brazilian vegetaction (GIANNOTTI and LEITAO FILHO 1992; MANTOVANI and MARTINS 1993; CASTRO *et al.* 1999; DURIGAN 1999). The Brazilian cerrado alone contains 25 genera of Rubiaceae (CASTRO *et al.* 1999), with *Alibertia* and *Psychotria* containing the greatest number of species.

Variations in chromosome number are useful for stablishing the limits of a species and for distinguishing among species within a group that shows extensive morphological and physiological variation or has a wide geographic distribution (PIEROZZI and MENDACOLLI 1997; BORTOLETI *et al.* 2002).

The usefulness of comparing chromosome numbers has been shown for the genus *Genipa* (Rubiaceae). Thus, ZAPPI *et al.* (1995) described a new species, *Genipa infundibuliformis*, based on individuals initially identified as *Genipa americana*. The species were differentiated based on morphological characteristics and their chromosome number, with 2n = 22 in *G. americana* and 2n = 20 in *G. infundibuliformis* (PIEROZZI and MENDACOLLI 1997).

There are few studies on the chromosomes of Rubiaceae, with most of the data available having been gathered by ROBBRECHT (1988). Most studies have dealt with *Coffea* (KRUG 1934; PINTO-MAGLIO and CRUZ 1988) and it has been suggested that the basic number for the family is x = 11 (KIEHN 1985).

In this study, we analysed the karyotype of 12 species of Rubiaceae from cerrado in southeastern Brazil (exception of *Genipa americana*) in order to assess the taxonomic and evolutionary position of the family as a whole.

MATERIALS AND METHODS

The species of Rubiaceae used were collected mainly in protected areas of cerrado in the state of São Paulo, which included the Experimental and Ecological Station of Assis (22°33'S and 50°22'W) and Itirapina

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(22°15'S and 47°49'W), the Biological Reserve and Experimental Station of Mogi Guaçu (Campininha Farm, 22°15'S and 47°08'W), the Experimental Station of Mogi Mirim (22°27'S and 46°57' W) and a disturbed area of the Agronomic Institute in Campinas (Santa Eliza Farm, 22°54'S and 47°05'W). Only one species was collected in a cerrado area in the state of Mato Grosso do Sul, at Três Lagoas (20°41'46"S and 51°40'54"W), located on the border with the state of São Paulo (Table 1).

The species were identified by a specialist and classified into subfamilies and tribes according to ROBBRECHT (1988). Voucher specimens were deposited in the UEC Herbarium (Department of Botany, Institute of Biology, UNICAMP).

For meiotic analysis, floral buds were collected and fixed in Carnoy solution (ethanol: acetic acid, 3:1, v/v) for 24 h and then stored in 70% ethanol, at 4°C. Slides were prepared by squashing and staining anthers with 2% aceto-carmine, as described by MEDINA and CONAGIN (1964).

For mitotic analysis, root tips were obtained from seeds germinated in petri dishes at 28°C. The roots were pre-treated with a saturated solution of paradichlorobenzene (PDB) for 5 h at 16-18°C or 0,002 M 8-hidroxyquinolin (8-Hq) for 5h at 14-15°C then fixed in Carnoy solution for at least 24 h and stored in 70% ethanol at 4°C. Root tips were subsequently squashed and stained with 2% Giemsa (GUERRA 1983) then mounted with Entellan.

Chromosomal counts in mitosis and meiosis were done on at least 10 cells for each species. The mitotic cells were drawn with a camera lucida to determine their size and centromeric index (CI) defined as the ratio between the short arm and the total length of each chromosome. The mean ideograms for each species were calculated. The total chromatin length (TCL), defined as the sum of the length of all of the chromosomes of a cell, and the TF% (a measure of karyotype symmetry), defined as the ratio between the total length of the short arms of all the chromosomes and TCL, were calculated according to HUZIWARA (1962). The morphological classification of the chromosomes followed the nomenclature proposed by GUERRA (1986).

RESULTS

The chromosome numbers of 14 species of Rubiaceae from cerrado belonging to two subfamilies (Ixoroideae and Rubioideae) were obtained, 10 of which have not been previously published (Table 2). The subfamily Ixoroideae was represented by six species and four genera of the tribe Gardenieae. All of the species had chromosome numbers that were multiples of 11, with most having 2n = 22, although one polyploid species had 2n = 66 (Table 2, Fig. 1).

In the subfamily Rubioideae, the chromosome number varied from 2n = 20 to 2n = 32. The chromosome numbers varied among the four tribes examined with 2n = 20 in Coccocypseleae, n = 11 (2n = 22) in Coussareeae, 2n = 22 (*Palicourea rigida* and *Psychotria hoffmannseggiana*) and 32 (*Psychotria deflexa*) in Psychotrieae and 2n = 28 in Spermacoceae (Table 2, Fig. 2).

The chromosomes varied in length from 1.26 µm in *Amaioua intermedia* to 7.33 µm in *Psychotria deflexa.* However, in most species the length ranged from 1.3 to 3.5 µm (Table 2). The largest TCL was observed in *Amaioua intermedia* (146.79 µm), because of its high chromosome number (2n = 66), but

Table 1 — Species of Rubiaceae, with information on the habits of the plants and their respective localities and collection numbers (AMC*); sh = shrub; tr = tree; he = herb.

Subfamily/ Tribe	Species	Habit	Locality	Collector number
IXOROIDEAE				
Gardenieae	*Alibertia concolor* (Cham.) K. Schum.	sh	Assis	02/64
	Alibertia edulis (L. C. Rich.) A. Rich. ex DC	sh	Mogi-Guaçu	02/50
	Alibertia sessilis (Vell.) K. Schum.	tr	Assis	01/38
	Amaioua intermedia Martius	tr	Itirapina	02/60
	Genipa americana L.	tr	Três Lagoas	01/30
	Tocoyena formosa (Cham.&Schltdl.) K.Schum.	tr	Campinas	02/68
RUBIOIDEAE				
Coccocypseleae	*Coccocypselum lanceolatum* (R. & P.) Pers.	he	Mogi-Guaçu	01/14
Coussareeae	*Coussarea hydrangeifolia* (Benth.) Müll. Arg.	tr	Mogi-Guaçu	01/15
Psychotrieae	*Palicourea rigida* H. B. K.	sh	Assis	01/20
	Psychotria deflexa DC.	sh	Mogi-Guaçu	02/48
	Psychotria hoffmannseggiana (R. & S.) M. Arg.	sh	Mogi-Mirim	01/01
Spermacoceae	*Borreria latifolia* (Aubl.) K. Schum.	he	Mogi-Guaçu	02/63
	Borreria verticillata (L.) G. F. W. Meyer	he	Itirapina	02/51
	Richardia brasiliensis Gomes	he	Campinas	01/05

* Collector number (Andréa Macêdo Corrêa).

Table 2 — Chromosome numbers and karyotype characteristics of the Rubiaceae species.

Subfamily/ Tribe	Species	n	2n	Chromosome length (μm)	TCL (μm)	TF%	karyotype formula
IXOROIDEAE							
Gardenieae	*Alibertia concolor*		22*	3,31 – 1,67	52,86	42,07	8m + 3sm
	Alibertia edulis		22*	2,79 – 1,36	44,39	42,89	10m + 1sm
	Alibertia sessilis		22	3,05 – 1,33	46,98	41,29	8m + 3sm
	Amaioua intermedia		66**	3,55 – 1,26	146,79	45,01	30m + 3sm
	Genipa americana		22	6,77 – 2,41	97,81	38,60	4m + 7sm
	Tocoyena formosa		22*	3,36 – 1,51	52,24	38,01	6m + 5sm
RUBIOIDEAE							
Coccocypseleae	*Coccocypselum lanceolatum*		20*	2,58 – 1,52	40,25	44,99	10m
Coussareeae	*Coussarea hydrangeifolia*	11**		—	—	—	—
Psychotrieae	*Palicourea rigida*	11*		—	—	—	—
	Psychotria deflexa		32*	7,33 – 2,77	140,46	40,06	7m + 9sm
	Psychotria hoffmannseggiana		22	5,08 – 2,63	81,75	42,38	10m + 1sm
Spermacoceae	*Borreria latifolia*		28*	2,96 – 1,49	60,25	43,87	14m
	Borreria verticillata		28	3,54 – 1,76	70,32	47,45	14m
	Richardia brasiliensis		28*	2,69 – 1,49	56,35	44,10	14m

Unpublished counts for the species * and for the genus * *, TCL = total chromatin length, TF% = kayotype symmetry; m = metacentric and sm = submetacentric.

the sizes of its chromosomes were similar to those of most of the other species. The smallest TCL was observed in *Coccocypselum lanceolatum* (40.25 μm), which not only had the lowest chromosome number (2n = 20), but also had the longest chromosome pair with the smallest length among the species studied (Table 2).

Metacentric chromosomes predominated in almost all of the species in which morphological characterization was possible, except for *Genipa americana*. No acrocentric and telocentric chromosomes were observed (Table 2, Figs. 3 and 4). The largest TF% occurred in *Borreria verticillata* (47.45), in which all of the chromosomes were metacentric. The smallest TF% values were obtained in *Genipa americana* (38.60) and *Tocoyena formosa* (38.01), species with 7 and 5 equal submetacentric chromosomes, respectively.

DISCUSSION

Karyotypic analysis - As pointed out by KIEHN (1985), little is known about the chromosomes of tropical and subtropical species of Rubiaceae. The chromosome numbers presented here (Table 2) agree with those reported in the literature and generally with the basic number (x = 11) for this family (DARLINGTON and WYLIE 1955). The polyploid species (*Amaioua intermedia*, 2n = 66) had a multiple of 11. The other numbers found (2n = 20 and 2n = 28) have already been reported for the same tribes or genus. The number 2n =32 had been reported for another tribe of the subfamily. Other basic numbers for this family include x = 2, 3, 4, 8, 9, 10, 12, 20 and 22 (ROBBRECHT *et al.* 1996).

Species of *Coffea* (PIEROZZI *et al.* 1999), and *Genipa americana* (PIEROZZI and MENDACOLLI 1997) are the only species of Rubiaceae with well known ideograms. PIEROZZI *et al.* (1999) showed that the chromosomes of *Coffea canephora* and *C. dewevrei* varied from 0.85 to 2.38 μm and from 0.83 to 2.03 μm, respectively. These sizes are similar to those of the smallest chromosomes described here (Table 2).

The centromeric position observed in this study also agrees with the literature, with a prevalence of metacentric and submetacentric chromosomes and no acrocentric or telocentric chromosomes. The ideograms of the species examined can be classified as symmetrical, as shown by the high values of TF% (> 38.01, Table 2). The maximum karyotypic symmetry corresponds to a TF% value of 50 (STEBBINS 1971).

Subfamily Ixoroideae - Among the six species of the subfamily Ixoroideae, tribe Gardenieae, three belonged to the genus *Alibertia* (*A. concolor*, *A. edulis* and *A. sessilis*). All had 2n = 22 and only for *A. sessilis* has the chromosome number already been reported (FORNI-MARTINS *et al.* 1995). The differences among the ideograms of the three species were small and reflected variations in chromosome size and morphology.

Amaioua intermedia was the only species of the tribe Gardenieae to show polyploidy (2n = 66). The chromosome number and degree of polyploidy were previously unknown for this genus. The highest numbers previously presented for this subfamily were 2n = 44 for *Ixora schawii* (tribe Pavetteae) (FAGERLIND 1937) and 2n = 66 and 88 in *Coffea ara-*

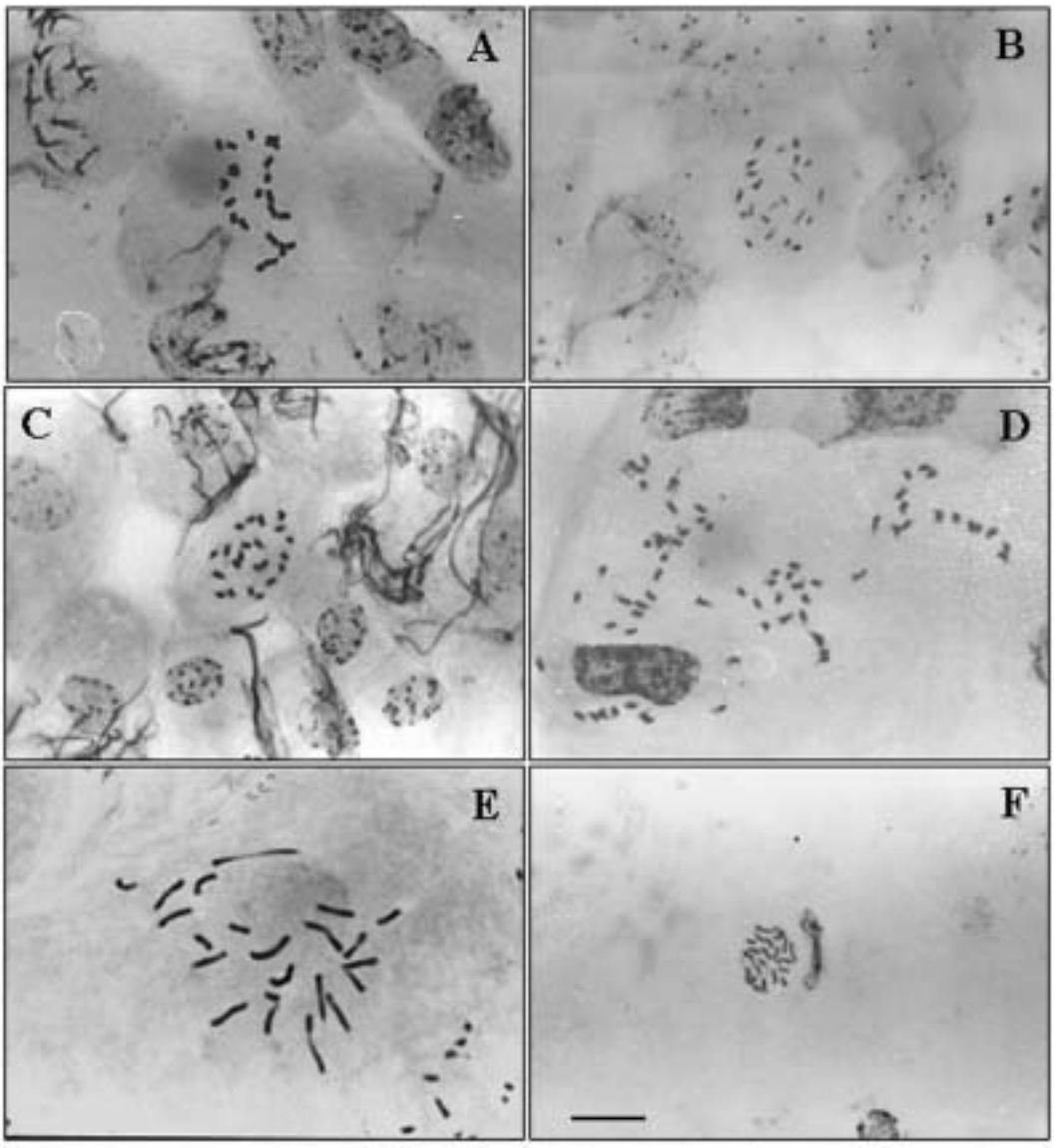

Fig. 1 — Chromosomes of the subfamily Ixoroideae: A) *Alibertia concolor* (2n=22); B) *A. edulis* (2n= 22); C) *A. sessilis* (2n=22); D) *Amaioua intermedia* (2n=66); E) *Genipa americana* (2n=22); F) *Tocoyena formosa* (2n=22). Bar 10 μm.

bica var. *bullata* (tribe Coffeae) (Sybenga 1960). Future studies with chromosome banding should clarify the origin of this ploidy. The size of the chromosomes of *A. intermedia* was very similar to that of other species of the genus (1.26 – 3.55 μm). Polyploidy is very common in arboreal species of Rubiaceae (Robbrecht 1988). However, of the five arboreal species studied (Table 1), only *Amaioua intermedia* was polyploid.

The value of 2n = 22 for *Genipa americana* agrees with previous reports for this species in other populations from the states of São Paulo (Pierozzi and Mendacolli 1997) and Pernambuco (Bortoleti *et al.* 2002). This species has the longest chromosomes in the subfamily (2.42 – 6.77 μm). The chromosome sizes for *G. americana* reported by Pierozzi and Mendacolli (1997) were smaller than those found here. This discrepancy may reflect the degree of chromosomal contraction associated with the phase of cell division used to obtain the chromosome measures, e.g., metaphase for Pierozzi and Mendacolli (1997) and prometaphase in the present work (Fig. 1).

The karyotypic formula of four metacentric and seven submetacentric chromosomes presented here also differed from that described by Pierozzi and Mendacolli (1997), who found three metacentrics and eight submetacentrics. The position of the submetacentric chromosomes in the ideogram presented by Pierozzi and Mendacolli (1997) also differed from that observed here.

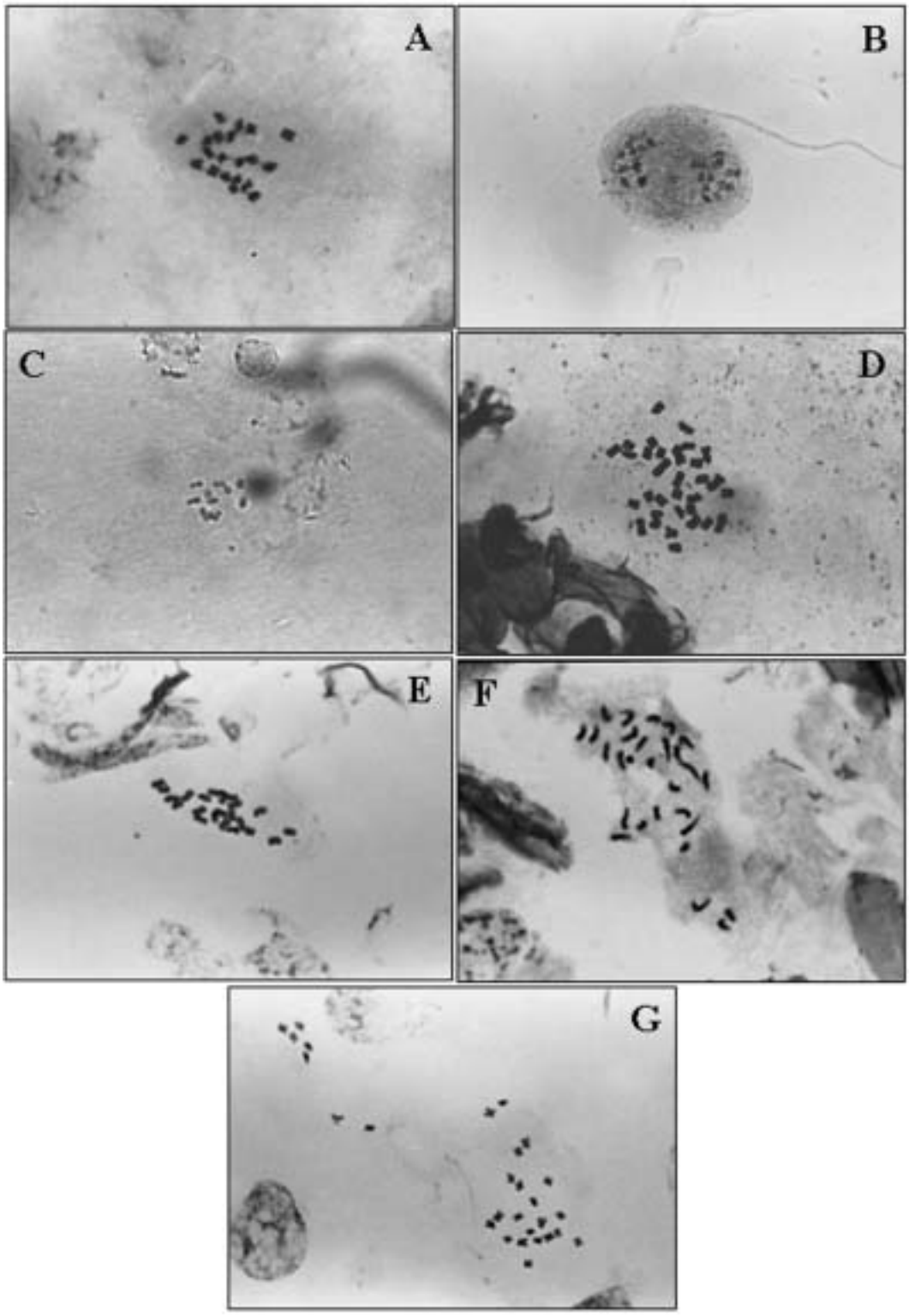

Fig. 2 — Chromosomes of the subfamily Rubioideae: A) *Coccocypselum lanceolatum* (2n=20); B) *Coussarea hydrangeifolia* (n=11); C) *Palicourea rigida* (n = 11); D) *Psychotria deflexa* (2n = 32); E) *P. hoffmannseggiana* (2n = 22); F) *Borreria verticillata* (2n = 28); G) *Richardia brasiliensis* (2n = 28). Bar 10 μm.

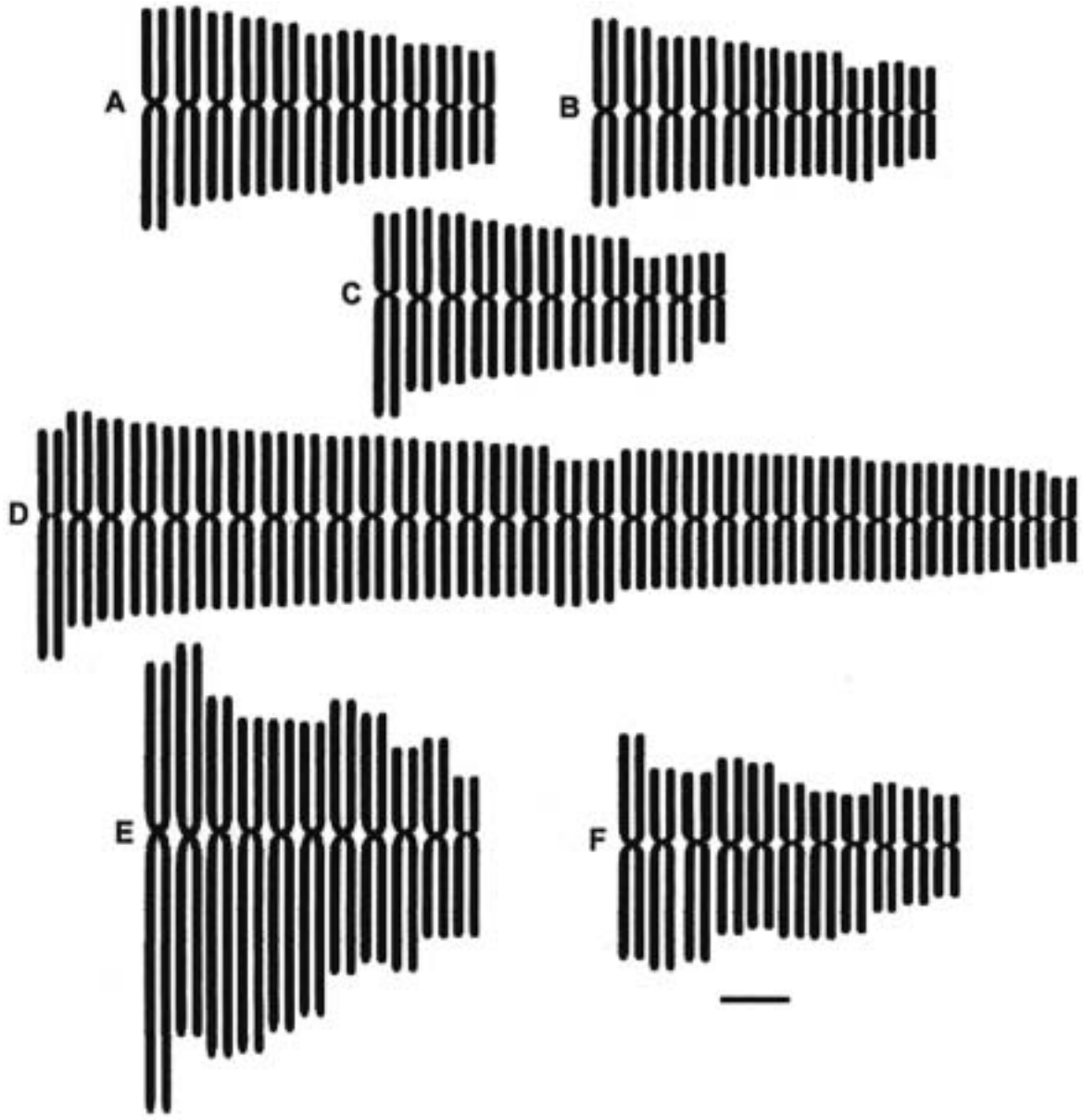

Fig. 3 — Ideograms of the subfamily Ixoroideae: A) *Alibertia concolor* (2n=22); B) *Alibertia edulis* (2n=22); C) *Alibertia sessilis* (2n=22); D) *Amaioua intermedia* (2n=66); E) *Genipa americana* (2n=22); F) *Tocoyena formosa* (2n=22). Bar 1 µm.

The count of 2n = 22 in *Tocoyena formosa* confirmed the chromosome meiotic number (n = 11) previously described by COLEMAN (1982).

Subfamily Rubioideae - The chromosome number observed for *Coussarea hydrangeifolia* (n = 11) is the first description for this species, genus and tribe, and agreed with the basic number for this family, x = 11 (DARLINGTON and WYLIE 1955).

The chromosome number of 2n = 20 for *Coccocypselum lanceolatum* (tribe Coccocypseleae) differed from the basic number of the family, but has also been reported for other species of the genus, including *C. brevipetiolatum* and *C. decumbens* (KIEHN 1985). Other numbers found in species of the this genus include 2n = 10-12 in *C. herbaceum*, 2n = 20-40 in *C. hirsutum* and 2n = 38-40 in *C. lumbellatum* (KIEHN 1985).

The tribe Psychotrieae shows considerable variation in the chromosome numbers of its genera and species, with 2n = 22 (n = 11) in most species, but high numbers in others, e.g. 2n = 88 and 2n = 132 in *Psychotria orophila* and *P. mahonii*, respectively (KIEHN 1985), and 2n = 110 + 1 in *Hydnophytum moseleyanum* (ROBBRECHT 1996).

The genus *Psychotria*, the most representative of the tribe, has chromosome numbers with n = 11, 12,

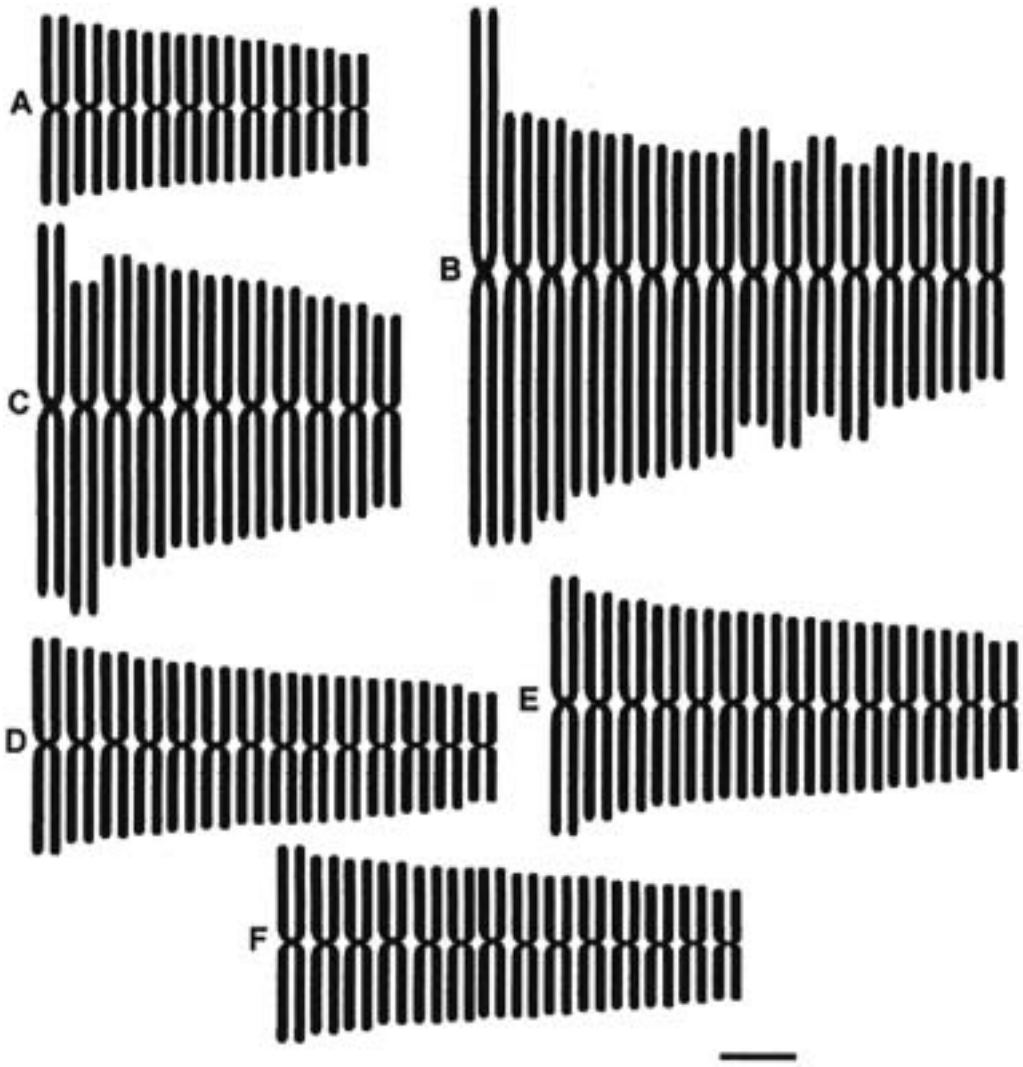

Fig. 4 — Ideograms of the subfamily Rubioideae: A) *Coccocypselum lanceolatum* (2n=20); B) *Psychotria deflexa* (2n=32); C) *Psychotria hoffmannseggiana* (2n=22); D) *Borreria latifolia* (2n=28); E) *Borreria verticillata* (2n=28); F) *Richardia brasiliensis* (2n=28). Bar 1 μm.

17, 22, and 34 (GOLDBLATT 1985; GOLDBLATT and JOHNSON 1991, 1994) and 2n = 22, 40, 44, 88 and 132 (BOLKHOVSKIKH *et al.* 1969; GOLDBLATT 1981; KIEHN 1985; GOLDBLATT and JOHNSON 1994; ROBBRECHT *et al*. 1996; PINTO-MAGLIO *et al*. 1997).

The value of 2n = 22 in *Psychotria hoffmannseggiana* confirmed the findings of PINTO-MAGLIO *et al.* (1997). On the other hand, the number 2n = 32 (n = 16) obtained here for *Psychotria deflexa*, has not previously been reported for this genus. *Psychotria deflexa* (2n = 32) had the largest chromosomes in this study (2.77 – 7.33 μm). The increase in the number of chromosomes in *Psychotria deflexa*, from the basic number of x = 11, probably occurred through the incorporation of chromosomes rather than through breaks in chromosomes already present in the genome.

The count of n = 11 in *Palicourea rigida* partially agreed with reports for other species of the genus, with *P. macgravii* having 2n = 22 + 3B (PINTO-MAGLIO *et al*. 1997). The chromosome numbers of *Psychotria hoffmannseggiana* and *Palicourea rigida* agreed with the basic number (x = 11) for the family (DARLINGTON and WYLIE 1955).

The three species of the tribe Spermacoceae, *Borreria latifolia*, *Borreria verticillata* and *Richardia brasiliensis*, had 2n = 28. This tribe also showed considerable variation in its chromosome numbers, with maximum values of 2n = 84 in two species of *Borreria*. There are several other species containing the same number of chromosomes as reported here (2n = 28), including members of *Borreria*, *Diodia*, *Mitracarpus* and *Spermacoce* (ROBBRECHT 1996). Within the genus *Borreria,* we can find n = 14, 14II, 26-27II,

28 and 28II (GOLDBLATT 1981), and 2n = 22, 28, 32, 40, 44, 56, 64, 84 (BOLKHOVSKIKH *et al*. 1969; GOLDBLATT 1981, 1985, 1988; GOLDBLATT and JOHNSON 1990, 1991). The chromosome number of other species of *Richardia* is unknown.

Except for the chromosome numbers, which differed among species, tribes and subfamilies of Rubiaceae, the ideograms were very similar, particularly in relation to the position of the centromere (mainly metacentric). The variation in chromosome size observed among some species, did not correlate with any particular group, since species with larger chromosomes occured in various subfamilies, e.g. *G. americana* in Ixoroideae and *P. deflexa* and *P. hoffmannseggiana* in Rubioideae. Thus, chromosome size and morphology are of little taxonomic use in this family.

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