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# CHROMOSOME SURVEY OF THE RUBIACEAE<sup>1</sup>

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## ABSTRACT

The present knowledge of karyological characters, including basic numbers, ploidy levels, chromosome structures, karyotypes, interphase nuclear types, and DNA contents, and the broad range of variation for most of these characters within Rubiaceae (excluding Rubieae) are summarized. These data are related to the presently existing systematic concepts of the family. The use of karyological characters is exemplified in some taxonomically critical groups: Subfamily Antirheoideae sensu Robbrecht appears heterogeneous with regard to basic numbers and chromosome structure; it contains chromosomally homogeneous tribes (e.g., Vanguerieae or Guettardeae), but also groups with internal variation (such as the tribe Knoxieae or the genus *Chiococca*). Available chromosome data of the tribe Cinchoneae sensu Robbrecht seem to be in accordance with its morphologically and molecular-based subdivision in three tribes: viz. Cinchoneae s. str., Calycophylleae, and Coptosapelteae. The tribe Chiococceae sensu Bremer consists of at least two distinct entities characterized by their chromosome morphology and basic numbers: the former tribe Condamineae, and the genera *Chiococca* and *Exostema*. A possible subdivision of *Rondeletia* is indicated by three different basic numbers ( $x = 9$ ,  $x = 10$ ,  $x = 11$ ) occurring in the genus, while chromosome data give no hints regarding the position of *Wendlandia* and *Sipanea* in the tribe Rondeletieae. The tribe Isertieae comprises genera with basic numbers of  $x = 9$ ,  $x = 10$ , and  $x = 11$ . Thus the tribal position of *Acranthera* ( $x = 10$ ), *Heinsia* ( $x = 11$ ), and *Mycetia* ( $x = 11$ ) cannot be decided on the basis of chromosome numbers alone. *Sabicea* has taxa with either  $x = 9$  or  $x = 11$ ; morphological studies are required to check the generic delimitations. The close relationship of *Pauridiantha* and *Urophyllum* is supported by karyomorphology and basic number. A possible origin of the *Spermacoce* out of the tribe Hedyotideae is discussed.

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With more than 10,000 species (Mabberley, 1987) the Rubiaceae are one of the larger families in the plant kingdom. Karyological studies in the family show a broad range of variation in chromosome numbers and other chromosomal parameters. Compilations of karyological data, however, are either relatively old (Fagerlind, 1937; Mendes, 1938), regionally restricted (Mangenot & Mangenot, 1958: West Africa; Philip & Mathew, 1988: southern India), or not widely published (Kiehn, 1986a). Relatively numerous counts are published for Africa, India (especially southern India), and New Zealand, and many unpublished data (by the author) exist for Central America and Madagascar. For most other tropical and subtropical areas of the world karyological information on Rubiaceae is scanty. Otherwise, recent chromosome counts of Rubiaceae are scattered in the literature and not easily accessible to taxonomists. Besides the work on the temperate Rubieae, which are not discussed in this survey, only few attempts of more

intensive chromosome studies on material of known origin related to larger systematic groups have been made (Hedyotideae: Lewis, 1962, 1965; Anthospermeae: Puff, 1986; *Paederia*: Kiehn, 1991).

Earlier interpretations of chromosome data related to evolutionary trends and classification of Rubiaceae mostly dealt with basic numbers and ploidy levels (e.g., Verdcourt, 1958; Lee & Rink, 1985; Philip & Mathew, 1988). They were based on different systematic groupings of Rubiaceae (Schumann, 1891; Verdcourt, 1958; Wagenitz, 1959; Bremekamp, 1966) because a comprehensive multidisciplinary systematic survey of tropical Rubiaceae has been lacking until recently (Robbrecht, 1988). In the present paper the subfamilial, tribal, and subtribal classification of the Rubiaceae largely follows Robbrecht (1988) but also considers more recent publications that discuss the circumscription and composition of Robbrecht's tribes (e.g., Andersson & Persson, 1991: Cinchoneae, Condamineae; Bremer, 1992: Chiococceae; Bremer

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& Struwe, 1992: several tribes; Puff & Robbrecht, 1989: Knoxieae; Robbrecht et al., 1991: Morindeae).

The present survey of karyological data in Rubiaceae (excluding Rubieae) is based on a compilation of all data available from the literature until 1993 and on unpublished data of the author. It is hoped that this study will stimulate a more intensive use of karyological data for phylogenetic and systematic considerations in the family.

#### MATERIALS AND METHODS

Original chromosome data reported in this paper are based on field fixations (\*) or fixations from plants cultivated at the Botanical Garden of the University of Vienna, Austria (H.B.V.), or at the National Tropical Botanical Garden in Kauai, Hawaii, U.S.A. (NTBG (\*\*): *Chiococca alba* (L.) Hitchcock \*\*: origin unknown, seeds from Major Howell Botanical Garden (1984), cult. in H.B.V. sub RR-814 (WU). *Chiococca bermudiana* S. Brown \*\*: Bermuda Island. Spittall Pond, 1978, *Phillips s.n.* (K, WU). *Chiococca coriacea* Mart. & Gal. \*\*: Mexico. Veracruz: Balzopote, 22 Mar. 1986, *D. Lorence 4972*, cult. in NTBG sub 880087 (PTBG, WU). *Pauridiantha paucinervis* (Hiern) Bremek. subsp. *lyalii* (Baker) Verdc. \*\*: Madagascar. Prov. Diégo Suarez: Massif du Marojezy, NW of Mantantenina village, 24 July 1985, *Puff et al. 850724-1/3* (WU). *Sipanea hispida* Benth. ex Wernh. \*\*: Brazil. Distrito Federal: Córrego Bananal (15°35'S, 47°55'W), *Kirkbride 5229* (US). *Thyranospermum diffusum* Champ. \*\*: China. Jiangxi: Mt. Jiulian, seeds from Botanical Garden Long Wu Lu Shanghai (1983-539) (WU, Herb. Botanical Garden Long Wu Lu Shanghai). *Urophyllum ellipticum* Thw. \*: Sri Lanka. About 5 km N of Gilimale on the road to Carney, S slope of Adam's Peak, 10 Feb. 1984, *Lorenz EL-100284-18/1* (K, WU). *Urophyllum zeylanicum* Thw. \*\*: Sri Lanka. Above Hakgala Sanctuary Botanical Gardens (about 6 km SE of Nuwara Eliya), 12 Feb. 1984, *Lorenz EL-120284-25/4* (K, WU).

Materials and methods for original data of DNA measurements are listed in Kiehn (1986a), with additional explanation in Kiehn (1991).

Suitable tissues and methods for obtaining reliable chromosome data for Rubiaceae (counts, chromosome morphology, DNA contents) are described in Kiehn (1986a, 1991). Due to the presence of tannins in many Rubiaceae, self-tanning effects are often encountered in chromosome fixations, resulting in prefixation and/or clumping of chromosomes, or influencing staining conditions (see

Greilhuber, 1988, for the discussion of these problems). Thus, Giemsa staining (of Guerra, 1983) or Feulgen staining (after hydrolysis in 5 N HCl for 50 min. at 20°C) is not possible in many cases. Often additional staining with aceto-carmine (2% in 45% acetic acid) at room temperature is required, especially when dealing with meiotic divisions.

Difficulties in obtaining proper karyological results due to small size and/or relatively high numbers of chromosomes can be (at least partly) avoided by a pretreatment of actively growing meristematic tissues with 8-hydroxy-chinolin (4-6 hr., 8-10°C) before fixation.

The basis for the discussion of chromosome numbers is a critical list of chromosome counts for Rubiaceae (excluding Rubieae) from original literature (Kiehn, 1986a), including more than 300 counts by the author. The checklist is continually updated, and will be published separately. It comprises about 2000 records for nearly 1000 taxa. It is sorted according to Robbrecht's classification and contains critical remarks on taxonomic and nomenclatural problems, and the reliability of deviating counts. The author welcomes suggestions and additions (e.g., unpublished counts, which can be added to the list). Excerpts of the actual list are available on request.

#### SURVEY OF ACTUAL KNOWLEDGE

##### CHROMOSOME NUMBERS

Due to determination problems and inconsistent nomenclature (both leading to the misnaming of investigated taxa), and also the difficulties in obtaining proper karyological results (many taxa having small chromosomes that clump together, tannins often interfering with fixation and/or staining procedures, etc.), older chromosome data for Rubiaceae must be looked at with caution. A critical checklist of chromosome counts in Rubiaceae (excluding Rubieae) contains chromosome data for about 10% of all Rubiaceae species (Kiehn, 1986a, unpublished). There are counts for at least one taxon of all tribes and subtribes of Robbrecht's classification except for Triainolepideae, Lathraeocarpeae, Aulacocalyceae, and Jackieae (Table 1).

Chromosome numbers in Rubiaceae (excluding Rubieae) range from  $2n = 12$  (e.g., in *Houstonia*) to  $2n = 220$  (or even higher) in *Coprosma* and *Lasianthus*, basic numbers from  $x = 6$  to  $x = 17$  (Table 1); a report of  $x = 29$  for *Hedyotis scandens* Roxb. (Sarkar et al., 1982) has to be confirmed. The predominant basic number is  $x = 11$  (many woody Rubioideae, Ixoroideae, Antirheoideae, Cin-

Table 1. Chromosome basic numbers and ploidy levels in the tribes and subtribes of Robbrecht's (1988) classification of Rubiaceae (except Rubieae) in subfamilial arrangement. Doubtful literature data are in parentheses.

	Investigated genera/taxa	Basic numbers	Ploidy levels
<b>1. CINCHONOIDEAE</b>			
Cinchoneae			
Cinchoninae	9/22	11, 13, 14, 17, (18)	2x, 4x, 6x
Mitragyninae	2/7	11	4x
Naucleaeae			
Adininae	4/5	11	4x
Neolamarckiinae	1/1	11	4x
Naucleinae	2/5	11	4x, 8x
Hillieae	1/1	17-18	2x
Henriquezieae	3/3	7?, 8?	4x?
Rondeletieae	6/17	9, 10, 11, (12, 14)	2x, 4x
Sipaneeae	1/2	11	2x, 4x
Condamineae	2/3	?11, 12	2x
Isertieae	8/47	9, 10, 11	2x, 4x, ?6x
Urophyllaeae	1/2	9	6x
Pauridiantheae	1/3	9	2x
<b>2. IXOROIDEAE</b>			
Gardenieae			
Diplosporinae	2/5	11	2x
Gardeniinae	27/64	(10), 11, 17	2x, 4x, 6x
Pavetteae	7/82	(10), 11	2x, 3x, 4x, 6x
Coffeeae	2/65	11	2x, 3x, 4x, 6x, 8x
Aulacocalyceae	—		
Hypobathreae	7/8	11, (12)	2x, 4x
<b>3. ANTIRHEOIDEAE</b>			
Retiniphyllaeae	1/2	?10	2x
Vanguerieae	12/35	11	(2x), 4x
Guettardeae	5/9	11	4x, 6x, 8x
Chiococceae	1/4	(11), 12, 13, 14	2x (4x)
Alberteae	2/2	?10, 11	4x
Cephalantheae	1/1	11	4x
Craterispermeae	1/1	11	2x
Knoxieae	4/12	10, (11, 12)	2x, 4x
<b>4. RUBIOIDEAE</b>			
Hedyotideae	24/167	6, 7, 8, 9, 10, 11, 13, 15, 17	2x, 4x, 6x, 8x, 12x
Ophiorrhizeae	1/10	11, (12)	2x, 4x
Coccocypseleae	1/6	10	2x, 4x
Argostemmataeae	1/3	11, 14	2x
Hamelieae	3/14	12, 14	2x, 4x, 6x, 8x, 12x
Schradereae	2/2	11	2x
Psychotrieae	12/127	(10), 11	2x, 4x, 6x, 8x, 10x, 12x
Triainolepideae	—		
Lathraeocarpeae	—		
Morindeae	4/16	(5), 11	2x, 4x, 8x, 20-22x
Coussareae	2/2	?11	2x
Paederieae	9/34	(10), 11, (12, 13)	2x, 4x, 6x, 8x
Anthospermeae			
Anthosperminae	5/62	11	2x, 4x, 6x, 8x
Coprosminae	3/79	(10), 11	(2x), 4x, 6x, 8x, 12x, 14x, 20x
Operculariinae	2/2	11	2x
Theligoneae	1/2	10, 11	2x
Spermacoceae	12/51	8, (10, 11), 14, 15, 17	2x, 4x, 6x

Table 1. Continued.

	Investigated genera/taxa	Basic numbers	Ploidy levels
Tribus incertae			
Catesbaeae	1/2	12	2x
Hippotieae	2/2	10?	4x
Tammsieae	1/1	?	4x
Jackieae	—		
Perameae	1/1	9	2x, 8x

chonoideae pro parte). Without other morphological or karyological information this number gives no hints for systematic groupings or relationships in Rubiaceae. Other basic numbers, however (from  $x = 6$ : *Houstonia*, up to  $x = 17$ : Cinchonoideae pro parte), may be useful for phylogenetic considerations (see discussion below).

Ploidy levels in Rubiaceae range from  $2x$  to  $20-22x$  (Table 1). While some taxonomic groups seem to be characterized by a certain ploidy level (e.g., Vanguerieae; see discussion below), polyploid series are often encountered in herbaceous groups like the Hedyotideae, but also occur in predominantly woody tribes such as the Psychotrieae. They sometimes seem to have originated in parallel fashion even within genera (e.g., *Anthospermum*: Puff, 1986; *Paederia*: Kiehn, 1991; *Psychotria*: Kiehn, 1986a).

Polyploidization of single cells ( $2x \rightarrow 4x$ , or  $4x \rightarrow 8x$ ) in seedling root tips has been observed several times (Kiehn, 1986a: 189, and literature cited there). Mitotic divisions of such cells are regular and lead to series of cells with the higher ploidy level, which also have larger cell volumes (and thus have chromosomes that are easier to count). Reports from such cells may be the reason for single  $4x$  reports concerning taxa normally reported to be diploid.

#### OTHER KARYOLOGICAL PARAMETERS

There are several notable features regarding Rubiaceae chromosome structures:

Normally, length and structure of all chromosomes within a diploid set are more or less uniform (Fig. 1: *Sipanea hispida* Benth. ex Wernh.); usually there are one or two pairs of satellite chromosomes. However, chromosome length and structure can differ considerably among rubiaceae taxa. Chromosomes can be characteristic for a given group or even for a taxon: members of the Vanguerieae, for example, have small compact chromosomes hardly exceeding a length of  $0.5 \mu\text{m}$  in

metaphase, while chromosomes of Pavetteae taxa might measure as much as  $3 \mu\text{m}$  in mitotic metaphase and are well structured longitudinally.

There are some remarkable exceptions to the uniformity of chromosome sets normally found in Rubiaceae; the implications of heteromorphic karyotypes in *Urophyllum* and *Pauridiantha* (Fig. 2) are discussed below.

Structural changes of the chromosomes during the cell cycle can be characteristic for a certain group; for example, Spermaceae have late condensation of comparatively large terminal (telomeric) parts of the chromosomes, a condensation type unusual in Rubiaceae, and can be distinguished from other Rubioideae by this character.

Karyotypes of Rubiaceae have been analyzed in some publications on the basis of conventionally stained material (e.g., Bhattacharyya, 1958: *Gardenia*; Sharma & Chatterjee, 1960: *Ixora*; both dealing with ornamental taxa). In these papers, secondary constrictions and structural characters of the chromosomes have been described and used to discuss relationships between the corresponding taxa. But the investigated chromosomes were not fully contracted (cf. descriptions and drawings of chromosomes). Thus these "structures" depend on the state of chromosome contraction and not primarily on their structural constitution, with the result that these studies cannot be reproduced.

Modern banding techniques have only been used in a few instances. Giemsa banding has been successfully applied to a few members of the Gardenieae (*Gardenia* sp., Kiehn, unpublished; *Genipa americana* L., Guerra, 1993, Kiehn, unpublished) while in other cases (Psychotrieae, Anthospermeae) these techniques failed, partly because of small chromosome size, partly due to the interference of tannins present in these groups (Kiehn, 1986a). Thus, the use of Giemsa sequential staining or other banding techniques for systematic purpose in Rubiaceae seems to be limited.

Data on interphase nuclear types of members of the family are very scanty (de Poucques, 1949;

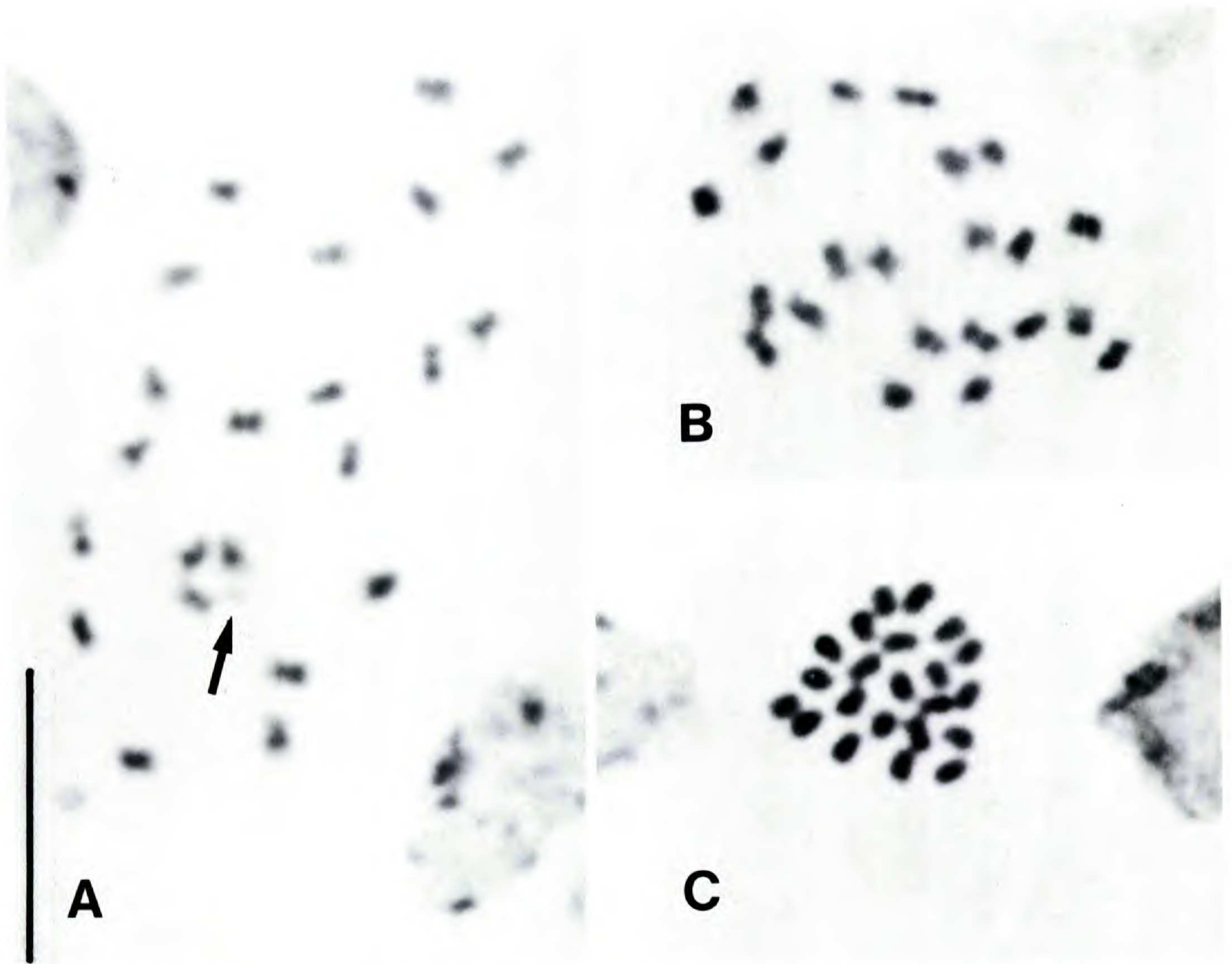


Figure 1. Mitotic chromosomes of *Sipanea hispida* (Kirkbride 5229):  $2n = 22$ .—A. Prophase.—B. Prometaphase.—C. Metaphase. Arrow indicates satellite. Bar = 10  $\mu\text{m}$ .

Kiehn, 1986a), but are useful to characterize certain groups (e.g., Spermaceae: Kiehn, 1986a).

DNA amounts for 49 species of Rubiaceae have been established (Kiehn, 1986a; Bennett & Smith, 1991; Cros et al., 1994). As would be expected from the small chromosome size of most Rubiaceae, these amounts are in the lower third of the range of angiosperm nuclear DNA values (cf. Bennett & Smith, 1976, 1991; Bennett et al., 1982). Between the lowest and highest 2C DNA amount of Rubiaceae there is a 9.6-fold range (Table 2). While whole groups (e.g., Vanguerieae) seem to be quite

uniform in their DNA amounts, DNA values can differ remarkably between taxa of the same basic number and ploidy level, suggesting both DNA increase (e.g., *Hamelia*, *Pavetta*) and DNA decrease (e.g., *Vanguerieae*, *Nauclea*).

#### IMPLICATIONS OF CHROMOSOME DATA

##### ANTIRHEOIDEAE SENSU ROBBRECHT (1988)

Table 3 summarizes the available chromosomal data known for tribes attributed to subfamily Antirheoideae. The only two counts for neotropical

Table 2. Lowest and highest known DNA values within Rubiaceae; cf. Kiehn (1986a). pg = picogram.

Total 2C-value	lowest	1.05 pg	<i>Serissa foetida</i>
	highest	10.13 pg	<i>Anthospermum spathulatum</i> subsp. <i>spathulatum</i>
1C-value per haploid genome (x)	lowest	0.1513 pg	<i>Vangueriopsis lanciflora</i>
	highest	0.7575 pg	<i>Hamelia patens</i>
Mean DNA-value per chromosome	lowest	0.0138 pg	<i>Vangueriopsis lanciflora</i>
	highest	0.0631 pg	<i>Hamelia patens</i>

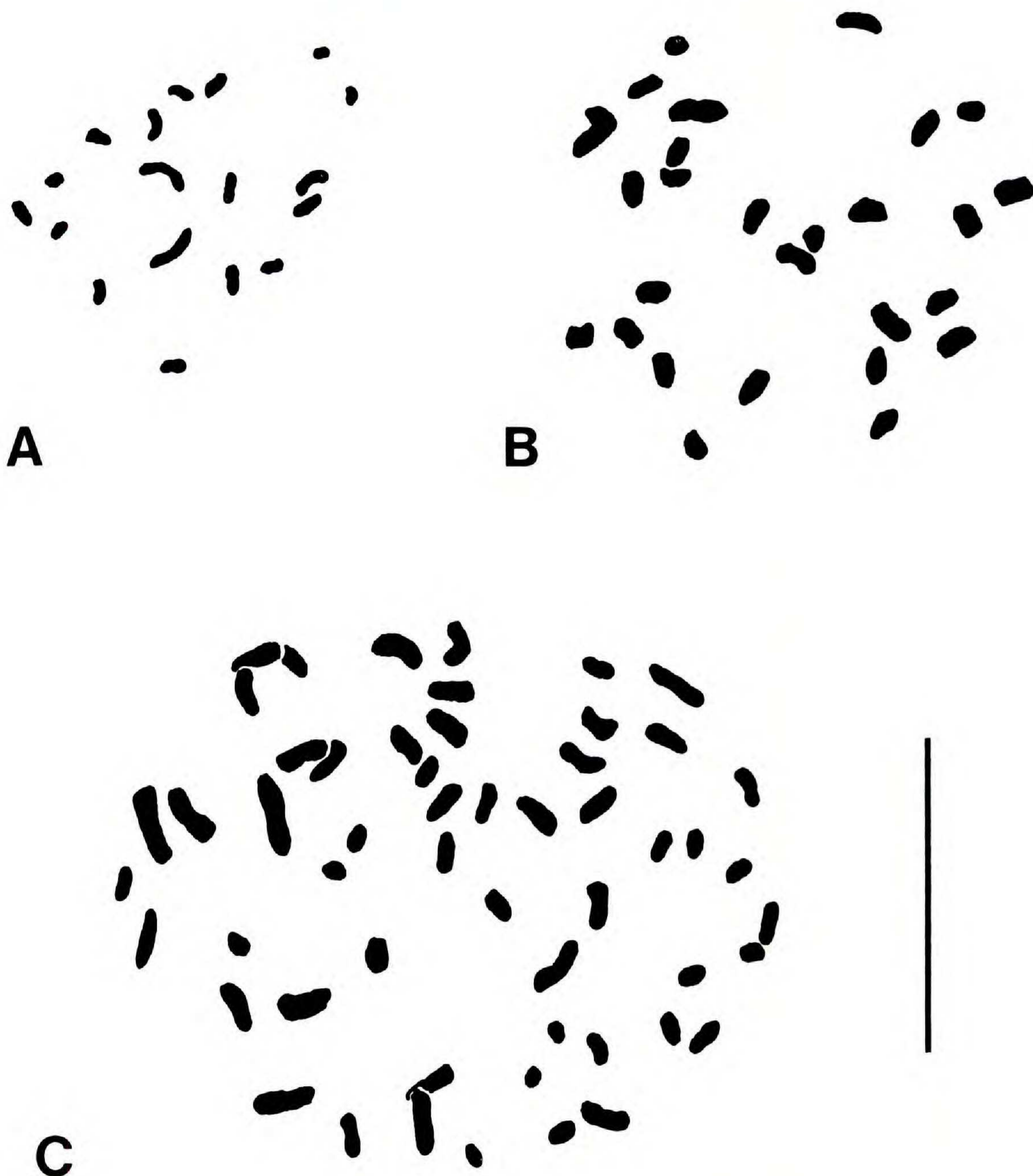


Figure 2. Chromosomes of :—A. *Pauridiantha paucinervis* subsp. *lyalii* (Puff et al. 850724-1/3):  $2n = 18$ .—B. *Urophyllum ellipticum* (Lorenz EL-100284-18/1):  $n = 27$ .—C. *Urophyllum zeylanicum* (Lorenz EL-120284-25/4):  $2n = 54$ . Bar = 10  $\mu\text{m}$ .

Retiniphyllae suggest  $x = 10$  on  $2x$  as ploidy level. On the other hand, Vanguerieae are comparatively well known cytologically. With one exception, all data indicate  $x = 11$  on a  $4x$  level. Therefore, the deviating taxon (*Cuviera acutiflora* DC.) should be reinvestigated. The Vanguerieae are uniform regarding other chromosome characters, such as chromosome length and structure, interphase nucleus structure, and DNA amount.

Taxa from five genera of Guettardeae have been investigated karyologically:  $x = 11$  is the general basic number; tetraploidy is found in four genera. Only the Hawaiian genus *Bobea* is represented by taxa on the hexa- and octoploid level. Chromosome structure and length differ from the Vanguerieae, mitotic metaphase chromosomes being more elongated and measuring nearly 1  $\mu\text{m}$ .

The taxonomic concept for the Chiococceae is under discussion. *Exostema* has been removed from the Cinchoneae and placed in the Condamineae by Andersson & Persson (1991). This view is supported by Bremer (1992), who included the Condamineae and *Exostema* in the tribe Chiococceae. Chromosomal data for Chiococceae sensu Bremer (1992) are summarized in Table 4. Unpublished counts by the author reveal  $2n = 28$  for *Chiococca bermudiana* and  $2n = 26$  for *C. coriacea*, while an exact number for *C. alba* could not be established ( $2n$  between 24 and 28). Literature data suggest  $x = 12$  on  $2x$ , and  $x = 11$  on  $4x$  level for this taxon. Chromosomes of *Chiococca* have prominent secondary constrictions and show some tendency to break at the centromeres. The chromosomes of *Exostema* are similar in structure to

Table 3. Chromosome basic numbers and ploidy levels in the tribes and genera attributed to the Antirheoideae sensu Robbrecht (1988). Doubtful literature data are in parentheses.

Genus [numbers of investigated taxa]	Basic number	Ploidy level
Retiniphyllae	?10	2x
<i>Retiniphyllum</i> [2]	?10	2x
Vanguerieae	11	(2x), 4x
<i>Canthium</i> [10]	11	4x
<i>Cuviera</i> [2]	11	(2x), 4x
<i>Keetia</i> [4]	11	4x
<i>Lagynias</i> [1]	11	4x
<i>Pachystigma</i> [1]	11	4x
<i>Peponidium</i> [1]	11	4x
<i>Plectronia</i> [2]	11	4x
<i>Psydrax</i> [2]	11	4x
<i>Pygmaeothamnus</i> [1]	?	min. 4x
<i>Rytigynia</i> [1]	11	4x
<i>Vangueria</i> [9]	11	4x
<i>Vangueriopsis</i> [1]	11	4x
Guettardeae	11	4x, 6x, 8x
<i>Antirhea</i> [1]	11	4x
<i>Bobea</i> [3]	11	6x, 8x
<i>Dichilanthe</i> [1]	11	4x
<i>Guettarda</i> [3]	11	4x
<i>Timonius</i> [1]	11	4x
Chiococceae	(11), 12, 13, 14	2x, (4x)
<i>Chiococca</i> [4]	(11), 12, 13, 14	2x, (4x)
Alberteae	?10, 11	4x
<i>Alberta</i> [1]	11	4x
<i>Nematostylis</i> [1]	10–11	4x
Cephalantheae	11	4x
<i>Cephalanthus</i> [2]	11	4x
Craterispermeae	11	2x
<i>Craterispermum</i> [1]	11	2x
Knoxieae	10, (11), 12)	2x, 4x
<i>Chorochoion</i> [1]	10	4x
<i>Knoxia</i> [2]	10, (11), 12)	2x, 4x
<i>Paraknoxia</i> [1]	10	2x
<i>Pentanisia</i> [8]	10	2x, 4x

the ones of *Chiococca*, and its reported basic numbers are  $x = 11$  (one count by Fagerlind, 1937, on material cultivated at Kew),  $x = 13$  (Kiehn, unpublished; McDowell, unpublished), and  $x = 14$  (McDowell, unpublished). *Portlandia* and *Cubanola* are characterized by large chromosomes and most probably have  $x = 12$  as the basic number. Thus, the tribe Chiococceae sensu Bremer (1992) is heterogeneous from the chromosomal point of view, and karyology provides distinctive characters: While *Portlandia* and *Cubanola* are doubtlessly closely related regarding their chromosome morphology, *Chiococca* chromosomes are different in structure. A possible relationship between *Chiococca* and *Exostema*, strengthened by the similar appearance of chromosomes and common unusual basic numbers, should be investigated further.

Results for Alberteae and Cephalantheae indicate tetraploidy on  $x = 11$ . The interphase nuclear type of *Cephalanthus* differs from the one characteristic for Vanguerieae and resembles the type of Rondeletieae, Sipaneeae, or Naucleaeae.

The situation for Knoxieae has become more diverse since the survey of Puff & Robbrecht (1989). Besides diploid and tetraploid representatives on  $x = 10$ , there are also reports with  $x = 11$  and  $x = 12$ . No data on chromosome morphology or interphase nuclei are available. More studies are needed to clarify the karyological situation in this tribe and its systematic implications.

In conclusion, the Antirheoideae sensu Robbrecht (1988) are heterogeneous as regards their karyological characters.

#### CINCHONEAE S.L.

In Table 5 the actual knowledge of chromosome data in genera placed in the Cinchoneae s.l., excluding those transferred to the Hedyotideae such as *Bouvardia*, is listed. Recently, Andersson & Persson (1991) emended Cinchoneae and Coptosapelteae and proposed a new tribe, Calycophyl-

Table 4. Chromosome numbers and ploidy levels of the Chiococceae sensu Bremer (1992). Doubtful data are in parentheses.

Genus [number of investigated species]	Basic number/ploidy level	Position sensu Robbrecht (1988)	Position sensu Andersson & Persson (1991)	Position sensu Bremer (1992)
<i>Chiococca</i> [4]	(11), 12, 13, 14/2x (4x)	Chiococceae	—	Chiococceae
<i>Cubanola</i> [1]	12/2x	Condamineeae	—	Chiococceae
<i>Exostema</i> [4]	11, 13, 14/2x	Cinchoneae–Cinchoninae	Condamineeae?	Chiococceae
<i>Portlandia</i> [2]	?11, 12/2x	Condamineeae	—	Chiococceae

Table 5. Chromosome numbers and ploidy levels of the Cinchoneae s.l. Doubtful data are in parentheses.

Genus [number of investigated species]	Basic number; ploidy level	Position sensu Robbrecht (1988)	Position sensu Andersson & Persson (1991)	Position sensu Bremer (1992)
<i>Calycophyllum</i> [1]	17; 2x	Cinchoneae-Cinchoninae (Cin-C)	Calycophylleae	—
<i>Cinchona</i> [5]	17; 2x (4x)	Cin-C	Cinchoneae (Cin)	—
<i>Coptosapelta</i> (incl. <i>Thysanospermum</i> ) [1]	10; 2x	Cin-C	Coptosapelteae (Cop)	—
<i>Cosmibuena</i> [1]	17-18; 2x	Cin-C	Cin	—
<i>Crossopteryx</i> [1]	11; 2x	Cin-C	Cop	—
<i>Exostema</i> [4]	11, 13, 14; 2x	Cin-C	Condamineeae?	Chiococceae
<i>Hillia</i> [1]	17-18; 2x	Hillieae	Hillieae	—
<i>Hymenodictyon</i> [3]	11; 2x; 4x; 6x	Cin-C	Cop	—
<i>Ladenbergia</i> [2]	17(-18); 2x	Cin-C	Cin	—
<i>Luculia</i> [3]	11; 4x	Cin-C	Cop	—
<i>Mitragyna</i> (incl. <i>Hallea</i> ) [2]	11; 4x	Cinchoneae-Mitragyninae (Cin-M)	Cop	—
<i>Remijia</i> [1]	17; 6x	Cin-C	Cin	—
<i>Uncaria</i> [5]	11; 4x	Cin-M	Cop	—

leae, based on a cladistic analysis of morphological characters. A basic number of  $x = 17$  is limited to their first clade including Cinchoneae and Calycophylleae (Table 6; the other genera of this clade have not yet been investigated karyologically). The division between the three tribes of this clade and the other taxa is supported by additional karyological data, such as structural differences between chromosomes, e.g., of *Cinchona* and *Hymenodictyon* (Kiehn, 1986a; Ehrendorfer, 1988: fig. 12).

The taxa in the emended tribe Coptosapelteae all have  $x = 11$  with ploidy levels ranging from  $2x$  to  $6x$  (Table 5), except one count for *Thysanospermum* (= *Coptosapelta*) *diffusum* Champ. with  $x = 10$ . This taxon probably does not belong to this clade (Robbrecht, pers. comm.).

RONDELETIEAE-SIPANEEAE (TABLE 7)

Within *Rondeletia* s.l., *Rondeletia* s. str. and its satellites *Rogiera* and *Arachnothryx* possibly are separated from each other by different basic numbers (Kiehn, 1986a). Whether  $x = 9$  really is characteristic for *Arachnothryx*,  $x = 10$  for *Rogiera*, and  $x = 11$  for the remaining taxa of *Rondeletia* should be verified by additional studies. As the report of  $x = 14$  and diploidy for a cultivated *Rondeletia* (Sarkar et al., 1982) is doubtful, all taxa of this complex apparently are tetraploids.

All investigated taxa of *Simira* and *Warszewiczia* are also tetraploids; their chromosomes are similar in structure. The strange number of  $2n = 42$  in *Simira* (Kiehn, 1986a) most probably is the

result of a fusion of two pairs of chromosomes of an original set of  $2n = 44$ .

*Wendlandia* is the only genus of the Rondeletieae (in this circumscription) with diploid taxa: it is also the only paleotropical one. Chromosomes of *Wendlandia* are generally smaller than those of the remaining Rondeletieae. These karyological data, however, are no help for the discussion of the placement of *Wendlandia* in Rondeletieae.

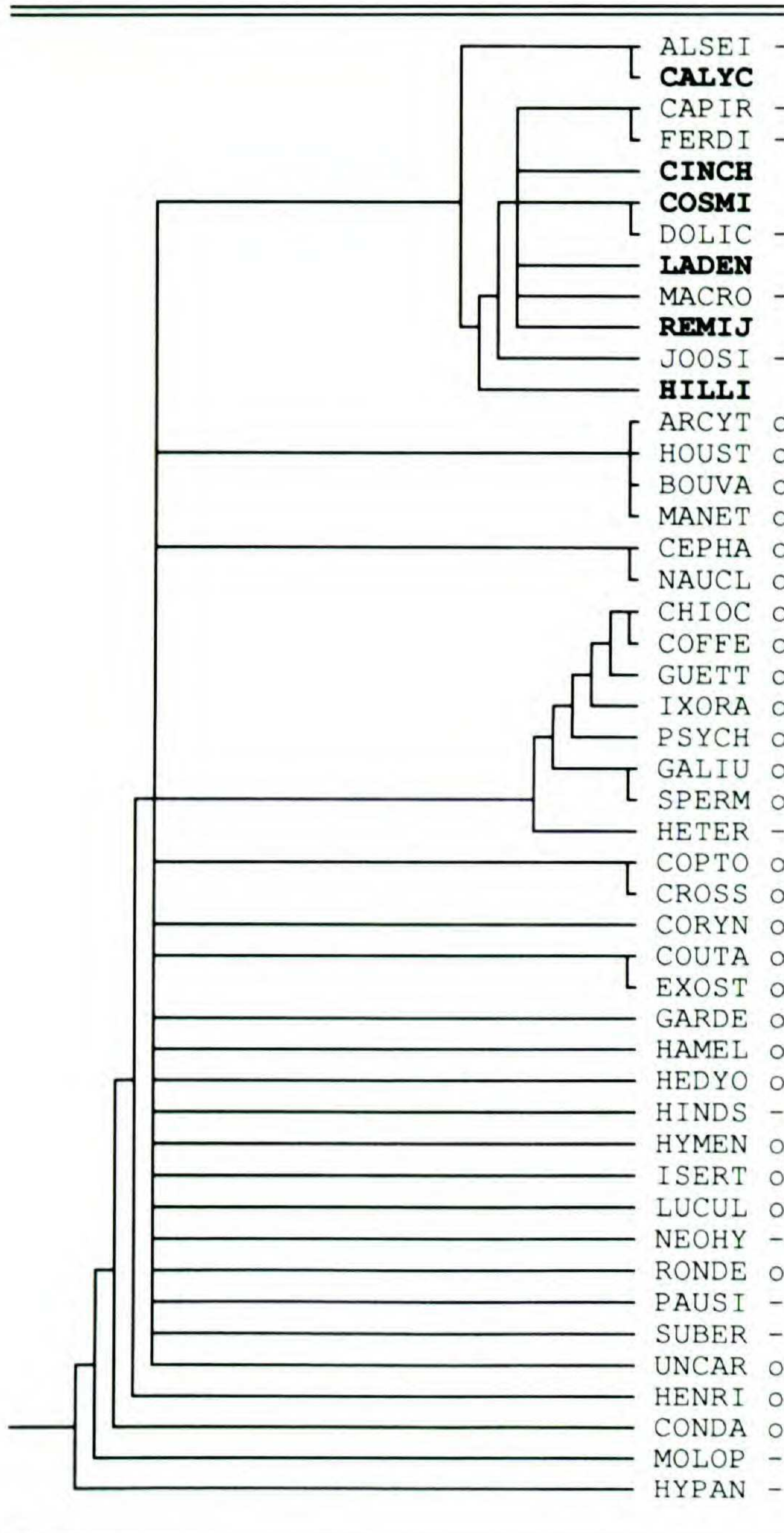
In *Sipanea*, one diploid and one tetraploid taxon on a basic number of  $x = 11$  are known. While chromosomes of mitotic metaphase (Fig. 1C) are not elongated as in Rondeletieae but rather compact as in Naucleaeae, interphase nuclear structures of *Sipanea* resemble those of both Rondeletieae and Naucleaeae.

ISERTIEAE (TABLE 8)

Different basic numbers occur in the Isertieae. The investigated genera with exclusively neotropical distribution, *Gonzalagunia* and *Isertia*, have  $x = 9$  and  $x = 10$ , respectively (Kiehn, 1986a, 1987), and are tetraploids. *Sabicea* occurs both in the Neo- and the Paleotropics. All its investigated taxa are tetraploids. In the Neotropics only species of *Sabicea* with  $x = 11$  have been found so far, while in the Paleotropics species with both  $x = 11$  and  $x = 9$  exist. Within a genus this is very unusual for non-herbaceous Rubiaceae and might indicate two different entities. Thus detailed morphological investigations are required to clarify the generic delimitation of *Sabicea*. The investigated taxa of



Table 6. Groups with  $x = 17$  in the strict consensus tree with hypothetical outgroup from the cladistic analysis of morphological characters by Andersson & Persson (1991). “—” not investigated, “O” other basic numbers.



the genera *Mussaenda* and *Pseudomussaenda* are diploids on a basic number of  $x = 11$ ; one literature record of  $2n = ca. 70$  for *Pseudomussaenda* obviously is wrong. The tribal position of *Mycetia* ( $x = 11, 2x$  and  $4x$ ), *Heinsia* ( $x = 11, 2x$ ), and *Acranthera* ( $x = 10, 2x$ ), currently included in Iseriidae, cannot be determined on the basis of chromosome numbers. *Acranthera*, however, has prometaphase chromosomes of ca.  $3 \mu m$  (Kiehn, 1986b: fig. 1c), which is unusually large for Iseriidae.

#### PAURIDIANTHEAE-UROPHYLLAE

*Urophyllum* and *Pauridiantha* share a karyological character not reported for any other Rubiaceae: they have a heteromorphic set of chromosomes. That means that both long and short chromosomes are present in the karyotype (Fig. 2). Together with their common basic number of  $x = 9$ , this might support the idea of a close relationship between the two genera as suggested by morphological studies (Buchner, 1995).

#### HEDYOTIDEAE-SPERMACEAE

Recent chloroplast DNA studies (Bremer et al., 1995; Natali et al., 1995) provide strong evidence for the Spermaceae having evolved out of the Hedyotideae, thus making the Hedyotideae paraphyletic. Karyological data could support this assumption: Hedyotideae reveal a broad diversity of chromosome basic numbers and ploidy levels, while Spermaceae predominantly have a basic number of  $x = 14$  and are diploids or tetraploids (Kiehn, 1986a). All recent models for the origin of  $x = 14$  in Spermaceae interpret this as a paleotetraploid state from basic numbers existing in Hedyotideae by loss or rearrangements of chromosomes: on a basis of  $x = 7$  by Majumdar (1975), who observed secondary associations of bivalents in

Table 7. Chromosome basic numbers and ploidy levels in the genera of the Rondeletieae and Sipaneeae of Robbrecht's (1988) classification of Rubiaceae. Doubtful literature data are in parentheses.

Genus [number of investigated species]	Basic number	Ploidy level
<b>RONDELETIEAE</b>	9, 10, 11, (12, 14)	2x, 4x
<i>Arachnothryx</i> [2]	9?	4x
<i>Rogiera</i> [2]	10?; 11?	4x
<i>Rondeletia</i> [2]	11; 14?	(?2x), 4x
<i>Simira</i> [2]	$2n = 42$ derived from $x = 11$	4x
<i>Warszewiscia</i> [1]	10-11	4x
<i>Wendlandia</i> [8]	11; 12?	2x
<b>SIPANEEAE</b>	11	2x, 4x
<i>Sipanea</i> [2]	11	2x, 4x

Table 8. Chromosome basic numbers and ploidy levels in the genera attributed to the Isertieae in Robbrecht's (1988) classification of Rubiaceae. Doubtful literature data are in parentheses.

Genus [number of investigated species]	Basic number	Ploidy level
<i>Acranthera</i> [1]	10	2x
<i>Gonzalagunia</i> [3]	9	4x
<i>Heinsia</i> [1]	11	2x
<i>Isertia</i> [3]	10	4x
<i>Mussaenda</i> [27]	11	2x
<i>Mycetia</i> [2]	11	2x, 4x
<i>Pseudomussaenda</i> [2]	11	2x, (6x)
<i>Sabicea</i> [8]	9; 11	4x

*Spermacoce* (however, no Hedyotideae with  $x = 7$  that are morphologically similar to Spermacoceae are known); or on a basis of  $x = 9$  by Kiehn (1986a), who considered the unusual numbers  $x = 17$  (in the African genus *Otiophora*, recently included in Spermacoceae by Igersheim & Rohrhofer, 1993),  $x = 16$  and  $x = 15$  (occasional in Spermacoceae) as possible intermediates.

The recently published basic number of  $x = 8$  on the diploid level in the neotropical genus *Galianthe* (Daviña & Cabral, 1991) offers another possibility for a paleotetraploid origin of  $x = 14$ : from  $2n = 4x = 32$  down to  $2n = 30$  (reported for the *Galianthe*-related genus *Triodon*, which has been sunk into *Galianthe* by Cabral & Bacigalupo, 1993) to  $2n = 28$ . This theory, however, would implicate a different origin for the  $x = 17$  of *Otiophora*, thus making the Spermacoceae polyphyletic.

#### CONCLUSIONS

In combination with the results of other research fields (e.g., molecular biology, morphology, and phytogeography), karyological characters have proven to be useful for understanding the relationships in taxonomically difficult groups of Rubiaceae. Moreover, they can help to explain evolutionary lines within the family. Therefore, published karyological data for Rubiaceae must be made more easily accessible for taxonomists and systematists, the exchange of information between different fields of research should be improved, and multidisciplinary research efforts on larger taxonomic groups should include chromosome studies.

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