REVIEW ARTICLE

CYTOLOGY IN THE SYSTEMATICS, PHYLOGENY AND EVOLUTION IN PLANTS IV. RUBIACEAE

P. M. MATHEW[1]* AND CINTHYA CHRISTOPHER[2]**

[1] Perakathuseril, Muttada P.O., Thiruvananthapuram 695 025
[2] Department of Botany, All Saints' College, Chackai, Thiruvananthapuram 695 007
** For correspondence. Email: cinthya_wilfred@yahoo.com

SUMMARY

A brief review highlighting the major cytological findings on the South Indian Rubiaceae is provided, considered in relation to the systematics, phylogeny and evolution of the family. The $x = 11$ is the stable and consistently occurring basic chromosome constitution in the family, which is considered as the earlier evolved one, arisen by secondary hybrid polyploidy involving ancestral $n = 6$ and 5. Most other basic lines are presumed to have arisen by descending/ascending aneuploidy from $x = 11$, while the $x = 17$ in *Cinchona* Linn. by tertiary hybrid polyploidy from $x = 11$ and 6. The phenomena of polyploidy and aneuploidy are not widespread in the family, except in the tribe Hedyotideae; and it appears that species diversification and evolution could have been mostly by the agency of cryptic chromosome structural alterations and/or by genetic changes. The intra- and interfamilial relationships and affinities of the family have been viewed in the light of cytological data. Bremekamp's treatment of various tribes appears cytologically tenable. His treatment of the tribe Hedyotideae, and suggestion of a monotypic tribe for *Ophiorrhiza* Linn. are appropriate. The gentianalean affinity proposed in most modern treatments as in the APG IV is commensurate with the cytological findings. The suggestion of a monotypic order, Rubiales is also worth considering.

INTRODUCTION

The Rubiaceae are a large family of predominantly woody genera comprising over 12000 species in 576 genera and 41 tribes included under 4 subfamilies such as Cinchonioideae, Dialypetalanthoideae, Antirheoideae and Rubioideae chiefly distributed in the warmer parts of the world (Mabberley 2017). The family includes several economically important plants like coffee, *Cinchona* and many ornamental plants. As regards the composition, systematic relationships and affinities of the Rubiaceae, there is great deal of discord. Despite the well recognized application of chromosome data in dealing with taxonomic ambiguities, phylogeny and evolution of related plant groups, relatively very little cytological data are available on the family. Notable earlier contributions are mostly on alien taxa, chiefly from North America (Lewis 1962a, 1966) and a very few from India (Raghavan & Rangaswamy 1941). An extensive study on the South Indian taxa comprising over 100 species in 28 genera and 15

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* Formerly Professor and Head of the Department of Botany, University of Kerala, Thiruvananthapuram.

tribes was carried out by the senior author in collaboration with a doctoral student, in the 1970s, and the results published in a series of papers (Mathew & Philip 1975, 1978, 1979, 1983, 1986 and Philip & Mathew (1975, 1976, 1987). There is little subsequent contributions on the group, and there is lacuna of cytological information of quite a number of taxa endemic to the South Indian region, which still remain unscreened, and there is much scope for future study of the group. The paper provides a brief review highlighting the major findings emerged from the South Indian study, considered in relation to the systematics, phylogeny and evolution of the family.

Basic chromosome number

The factor of basic chromosome number at the level of genera, tribes and families has played a notable role in shaping the prevailing concept of evolution. This has been one of the widely used parameters in formulating phylogenetic speculations, and also employed as a dependable and stable marker of the direction of evolution. Stebbins (1950) proposed diminution of basic number as playing significant role in the process of evolution in plants. Studies in many angiosperm families of the South Indian region have yielded convincing evidence in favour of this contention. Jones (1970), while agreeing with the reduction phenomenon which occurs more frequently, however, has emphasized that reversibility of the process is also in vogue. Polyploidy often influences and plays marked role in the evolution of basic numbers of the higher order producing unrelated basic numbers. A perusal of the available chromosome data shows that $x = 11$ is the deep-rooted and stable basic chromosome constitution in the family, occurring consistently in almost all the cytologically known tribes and genera (Table1), existing singly or in combination with its mostly descending and also ascending derivatives. In a few tribes (Hedyotideae, Gardenieae, Anthospermeae, Galieae), both ascending and descending numbers occur, of which Hedyotideae shows remarkable degree of aneuploid reduction series from $x = 11$ ($x = 10, 9, 8, 7$ and 6) together with an ascending of $x = 13$. Large number of genera are monobasic with $x = 11$.

Regarding the possible origin of the $x = 11$ constitution, there are two postulations, (1) by Sharma & Chatterjee (1960) who assumed the $x = 11$ arisen by amphidiploidy of two ancestral diploid taxa with $n = 6$, followed by aneuploid reduction to $n = 11$, and (2) by Lewis (1962b) who postulated that the $x = 11$ state may have originated by mixing of two ancestral, one with $n = 6$ and the other $n = 5$ straightaway forming $n = 11$. The presence of secondarily associated bivalents at meiotic metaphase I in several species of *Ophiorrhiza* studied from South India appears to favour this possibility. In one of the species, five 2-bivalent associations and an unassociated bivalent were reported (Mathew & Philip 1978), and this is suggestive that the haploid complement of the species contains two basic genomes. The question as to which of the two possibilities referred to above had really operated is a matter of reasoning. As already pointed out, the $x = 11$ in the family is deep-rooted, occurring in almost all the tribes, while the $x = 12$ state only sparsely present. Moreover, the $x = 11$ constitution has evolved in striking degree both in polyploid and aneuploid directions. The occurrence of a regular series of polyploids on $x = 11$ in the genus *Galium* (Fedorov 1969, Goldblatt 1984), and the existence of a line of descending series from $x =$

TABLE 1: Generawise chromosome data of the South Indian Rubiaceae, and tribewise basic numbers and ploidy in the cytologically known taxa of the family.

Tribe	Genus	Chromosome number		Basic number	Ploidy
		n	2n		
Cinchoneae				**9, 11, 17**	**2x, 4x**
	Cinchona L.	17	34	17	
	Luculia Sweet	22	44	11	
Naucleeae				**11**	**2x**
Rondelletieae				**10, 11**	**2x, 4x**
	Rondeletia L.	22	-	11	
Hedyotideae				**6, 7, 8, 9, 10, 11, 13**	**2x, 4x, 6x, 8x**
	Dentella Forst.	18	36	9	
	Pentas Benth.	10	20	10	
	Oldenlandia L.	9, 18, 27, 36	-	9	
	Anotis DC.	18	-	9	
	Ophiorrhiza L.	11	-	11	
Mussaendeae				**9, 11**	**2x, 4x**
	Mussaenda L.	11	-	11	
Hamelieae				**11, 12**	**2x, 4x**
	Hamelia Jacq.	12	-	12	
Gardenieae				**10, 11, 12, 17**	**2x, 4x**
	Chomelia L.	11	-	11	
	Gardenia Ellis	11	-	11	
Guettardeae				**11**	**2x**
	Guettarda L.	22	-	11	
Knoxieae				**10**	**2x, 4x**
	Knoxia L.	10	-	10	
Chiococceae				**11, 12**	**2x, 4x**
Vanguerieae				**11**	**2x, 4x**
	Plectronia L.	22	-		
Ixoreae				**10, 11**	**2x, 3x, 4x**
	Ixora L.	11	22	11	
	Pavetta L.	11	22	11	
Morindeae				**11**	**2x, 4x**
	Morinda L.	11	22	11	
Psychotrieae				**10, 11**	**2x, 3x, 4x**
	Psychotria L.	11	22	11	
	Chassalia Comm. ex Poir.	11	-	11	
	Geophila Don.	22	-	11	
Anthospermeae				**9, 10, 11, 12**	**2x, 4x, 6x**
	Serissa Commeris	11	-	11	
Spermacoceae				**14, 15, 16**	**2x, 4x, 8x**
	Spermacoce Dill.ex L.	28	-	14	
	Richardia Houston ex L.	14, 28	-	14	
Galieae				**10, 11, 12**	**2x, 3x, 4x, 6x, 20x**
	Rubia L.	11	-	11	
	Galium L.	11	-	11	

11 in the North American Hedyotideae are obvious evidence for considering the x = 11 state as the earlier evolved condition, because such extent and profusion of x = 11 may be clear evidence of its antiquity. As an earlier evolved constitution, the x = 11 has had a longer period of time to produce and establish functional polyploids and aneuploids in contrast to the x = 12. Based on this possibility, a tentative scheme of evolution of different basic constitutions existing in the family is proposed (Fig. 1). According to the scheme, the other basic numbers are considered to have evolved in different lines such as (1) by ascending aneuploidy resulting in x= 12 and 13, (2) by descending aneuploidy resulting in x = 10, 9, 8, 7, 6, (3) by both ascending and descending aneuploidy as in the tribes Gardenieae, Hedyotideae and Galieae, (4) by secondary hybrid polyploidy involving taxa with x = 8 and 7 resulting in x = 15 from which x = 14 and x = 16 arose by descending and ascending aneuploidy as in the tribe, Spermacoceae and (5) by tertiary polyploidy resulting in the x = 17 state as in the genus *Cinchona* of Cinchoneae. The n = 17 species, *Cinchona ledgeriana* Moens. reported from South India (Mathew & Philip 1979) showed varying numbers of 3-bivalent and 2-bivalent associations suggestive of its complement containing three basic genomes implying that the taxon could be an allohexaploid originated by mixing of n = 11 and n = 6 progenitor taxa. In view of the primitive position of the tribe, Cinchoneae, it may be possible that the x = 17 constitution in the genus may have evolved during the early diversification of the family.
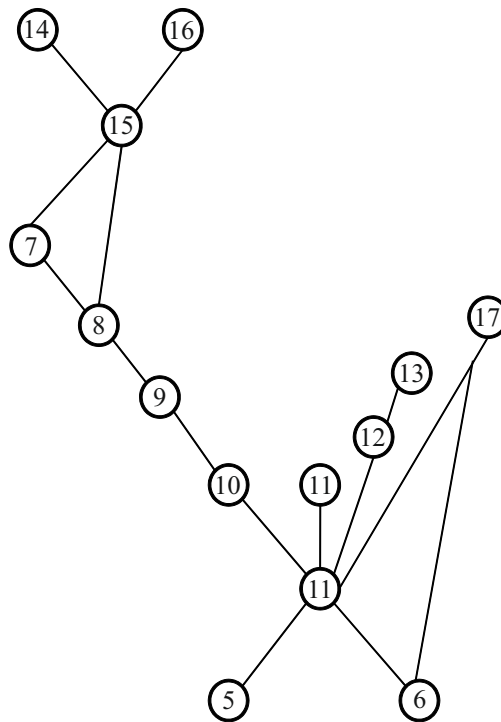

Fig. 1: Tentative scheme of evolution of basic chromosome numbers in Rubiaceae.

Polyploidy

Polyploidy is known to be the most widespread cytogenetic process which has greatly influenced the evolution of higher plants (Stebbins 1971). The phenomenon potentially has important ecological and evolutionary consequences, and generally viewed as an important driver of plant evolution, and also believed to be the single most important mechanism of sympatric speciation of land plants (Beest et al. 2012). Polyploidisation can alter plant morphology, phenology and physiology generating invasive potential by which can cope with fluctuating environments, and expand to new niches (Parisod et al. 2010). In cases where more than one cytotype occur naturally in native ranges, the polyploids thrive better. In the Rubiaceae, there are more than 45% of the cytologically known species which are polyploids, and this much of polyploidy apparently suggests that the phenomenon has been a major factor in the evolution of the family. However, the data of polyploidy and the levels of ploidy in the South Indian group indicate that the phenomenon has not been widespread in the region. In most genera, globally, the commonest ploidy is 4x, and higher levels known in a very limited number of genera of Hedyotideae and Galieae. In the South Indian group, polyploids occur only in 12 genera out of the 28 studied, of which *Oldenlandia* (Hedyotideae) is the only genus showing ploidy levels higher than 4x. Among the 11 species of the genus, seven were polyploids constituting a polyploid series on x = 9 running up to 8x. The restricted occurrence of polyploid taxa among the members indigenous to this region draws striking contrast with other angiosperm families and pteridophytes studied from the region.

Stebbins (1971) pointed out growth habit as a factor which influences the frequency of polyploids in angiosperms such that higher frequency in perennial herbs, lowest in annuals and intermediate in woody plants. But this appears to be the other way round in the rubiaceous taxa of South India in which polyploidy abounds in herbaceous taxa as in genera like *Hedyotis, Oldenlandia* etc. An apparent exception is the woody genus *Psychotria* in which majority of the species are tetraploids. The real reason for the rarity of polyploids in woody angiosperms is not clearly understood. Stebbins' (1971) contention is that in cases of more advanced families with homogeneous chromosome numbers, the absence of well developed polyploidy may be due to their selective disadvantage during their recent evolutionary history. He holds that, during the initial diversification and expansion of such families, there could have been a great burst of polyploidy associated with hybridization, and that the higher basic numbers including x = 11 and 10 in them are of secondary hybrid polyploid origin. The postulated origin of the stable x = 11 in the Rubiaceae, and also x = 17 occurring in certain woody genera like *Cinchona* assumes importance in this context. In view of the widespread occurrence of x = 11 in the Rubiaceae, it may be reckoned that extensive polyploidy on x = 11 following natural hybridization must have taken place during the early evolution of the family, and that in the later period of diversification and speciation, the polyploidy phenomenon apparently played only a lesser and restricted role.

Concerning the type of polyploids and the mode of their origin in the Rubiaceae, previous workers have projected different suggestions such as (1) Fagerlind (1937) reported natural segmental allopolyploidy in species of *Galium*, (2) Raghavan & Rangaswamy (1941), based on their findings in Indian members, suggested that allopolyploidy played a significant role in speciation in the family, (3)

Stebbins (1971) has reported natural allopolyploids in species of *Galium* and (4) Lewis & Terrel (1967) suggested autopolyploidy as an essential feature in evolution of American species of *Hedyotis*. Great many of the South Indian species of the family are tetraploids showing normal meiotic behavior, which is so even in the tetraploid and octaploid species of *Oldenlandia*. This may be suggestive of all their being allopolyploids. However, in certain species of *Oldenlandia* (*O. auriculata, O. pruniosa*) there was evidence suggestive of their autopolyploid or segmental allopolyploid nature.

Intraspecific polyploidy

The occurrence of more than one cytological type within previously recognized taxonomic species has been known in many plant groups. In the Rubiaceae, intraspecific polyploidy has been reported in species of over a dozen genera, of which the situation in the herbaceous genera like *Galium*, (Fagerlind 1937), *Hedyotis* and *Oldenlandia* (Lewis 1966) are more prominent. In the South Indian group, intraspecific polyploidy was rare, the only species being *O. umbellata* Linn. (Philip & Mathew 1987) which occurs here as diploid and tetraploid on x = 9. No difference in external features was noticed between them. In the case of a few taxa eg. species of *Cinchona, Pentas*, etc., all occurring here as diploid forms, their polyploid cytotypes are known from other geographical regions of which the situation in *O. corymbosa* Linn. is striking in as much as it occurs in three cytotypic forms (2x, 4x, 6x) in different continents (Lewis 1966).

Aneuploidy

Aneuploidy is known to occur in angiosperms mostly at the diploid level. In the best known cases, they involve an increase of one chromosome at a time; and this may be brought about by various karyological mechanisms. The trends of aneuploid changes are in ascending/descending directions, sometimes both occurring in the same genus. Chromosome data in the Rubiaceae show that although the x = 11 is the earlier evolved basic constitution, other aneuploid numbers derived from this occurs only in a limited number of tribes (Table 1), and in large bulk of genera, the x = 11 is conserved with considerable constancy, which appears suggestive that species differentiation in the family has occurred largely without changes in chromosome number. Of the few genera showing aneuploid derivations of x = 11, the genus *Hedyotis* presents a striking case of gross change of chromosome numbers taken place at the diploid level all occurring in the same geographical regions of North America (Lewis 1962a). Aneuploidy at polyploid level is of restricted occurrence in the family, most known cases being in the genus *Galium* in which Kliphuis (1967) reported a series of chromosome numbers (2n = 61, 62, 63, 64 and 65) which are aneuploid derivatives from the 6x (2n = 66) constitution. Among the South Indian group, intraspecific aneuploidy at polyploid level was incident in only one species (*Borreria hispida* K.Sch.) which occurs here in two aneuploid lines with 2n = 27 and 26, and the two forms differ from one another in a line of morphological features, which can be attributed to chromosomal difference.

From the foregoing considerations of polyploidy and aneuploidy, it is apparent that both the phenomena have played, but a less significant role in the evolution of the large bulk of tribes and genera in the family, especially the woody ones. It may, therefore, be possible that speciation in the family could

have been mostly through the agency of structural changes of chromosomes and/or genetic changes. Although detailed information of chromosome structural changes in the family is little known, there are certain indirect evidences suggestive of cryptic structural differences of chromosomes in a few diploid species of *Galium, Mussaenda, Pentas* etc. Stebbins (1971) has pointed out that high degree of pollen abortion in diploid species showing normal meiosis is suggestive of cryptic structural hybridity in them. This assumption is based on the possibility that parental species differing with respect to small chromosome repatterning would not be expected to display multivalents at meiosis.

Systematics

The family Rubiaceae has been subjected to a variety of treatments in respect of its composition and systematic relationships and affinities. Of the different classical treatments, the Bentham & Hooker's (1873) is the most comprehensive, while of the semimodern ones the Bremekamp's (1966). Between these and other more modern (APG IV 2016, Mabberley 2017) treatments, there are several points of agreement and also certain disagreements, especially concerning the subdivision, tribal content and systematic position of some of the tribes, and these are viewed here from the cytological perspective. Concerning the composition of the family, there is notable discord between the two most modern treatments viz. Mabberley (2017) and APG IV (2016). While Mebberley described the family under four subfamilies such a Cinchonoideae (10 tribes), Dialypetalanthoideae (7 tribes), Antirheoideae (8 tribes) and Rubioideae (16 tribes) with a total of 41 tribes, in the APG IV system there are only 3 subfamilies (Cinchonoideae, Ixoroideae, Rubioideae) with only 13 tribes. In the Bremer & Eriksson (2009) classification also there are only three same subfamilies as in APG IV, but with 44 tribes.

Tribes Naucleeae and Cinchoneae

In the Bentham and Hooker's (1873) treatment, the tribe Naucleeae occupies the most primitive position in the family, and the tribe Cinchoneae placed next to this. Bremekamp (1966), although considering both the tribes primitive, has assigned Naucleeae a position next to Cinchoneae. In the most modern (Mabberley 2017) treatment also, the Cinchoneae is given the primitive position. Bremekamp has reshuffled certain genera of Bentham & Hooker's Naucleeae (*Anthocephalus, Stephagyne, Cinearia*) placing them in the Cinchoneae. All the cytologically known genera of the two tribes (except *Cinchona*) have the same basic chromosome constitution of $x = 11$, and also similar pollen (bicelled) condition (Mathew & Philip 1986), and palynological similarities (Mathew & Philip 1983), and hence, Bremekamp's separation of these genera from Naucleeae to Cinchoneae appears untenable. Moreover, the genus *Cinchona* with $x = 17$, stands out from the rest of the genera of the tribe which are all $x = 11$ basic, and hence, it may be desirable to treat *Cinchona* under a monotypic tribe, occupying a primitive position in the family, as proposed by Philip & Mathew (1987). In the most recent APG VI (2016) and Mabberley (2017) treatments also, this has been assigned a similar primitive position, considering it in a separate subfamily, Cinchonoideae.

Tribe Hedyotideae

There is considerable disagreement between Bentham & Hooker (1873) and Bremekamp (1966) concerning the delineation of the Hedyotideae. According to Bremekamp (1966), the typical Hedyotideae should comprise only genera with valvate aestivation of the corolla lobes, plurilocular ovary cells, peltate placentation attached to the middle of the dissepiment; and on this ground, he has separated all the taxa of the tribe which are devoid of these attributes, placing them under different tribes. It may be noted that the Bentham & Hooker's (1873) Hedyotideae constitute cytologically a highly heterogeneous group with a wide range of basic numbers ($x = 6, 7, 8, 9, 10, 11$ and 13), the like of which does not exist in any other single tribe of the family. As regards the pollen nuclear number also this is the only tribe which is heterotypic in the entire family (Mathew & Philip 1986). Moreover, all the genera retained by Bremekamp in the Hedyotideae (*Dentella, Pentas, Hedyotis, Oldenlandia*) are chromosomally distinct from the other genera separated by him. He has also created a new tribe Ophiorrhyzeae. All the South Indian species of *Ophiorrhiza* are with $x = 11$ as basic number which is not present in any other genera separated by Bremekamp. Mathew & Philip (1987) also have recommended a monotypic tribe for *Ophiorrhiza* on account of the unique and characteristic consistent presence of 'pollen bud' formation in all the species of *Ophiorrhiza* during the development of the male gametophyte (Philip & Mathew 1975). In Mabberley's (2017) treatment, the genus *Ophiorrhiza* is separated from the tribe Hedyotideae, giving it a tribal status, placing it next to the Hedyotideae in the subfamily Rubioideae. Concerning the systematic position of the Hedyotideae also there is some discord. While Bentham & Hooker (1873) have placed the tribe in their Series A, both Bremekamp's (1966) and Mabberley's (2017) Hedyotideae occupy a very advanced position in their treatments. This appears very much commensurate with the cytological evidence of evolved chromosome constitution of the taxa of this tribe.

Tribe Mussaendeae

Only two genera of Bentham & Hooker's (1873) Mussaendeae are cytologically known, of which *Mussaenda* is $x = 11$ basic and the other, *Urophyllum* is $x = 9$ basic. Bremekamp (1966) has subdivided the tribe, assigning separate tribal status to both as Mussaendeae and Urophylleae, and in view of the clear chromosomal difference, the division appears appropriate.

Other tribes

Concerning the treatment and placement of the other tribes, especially Guettardeae, Knoxieae, Vangurieae, Ixoreae, Morindeae, Psychotrieae, Anthospermeae, Spermacoceae, Galieae, there is close agreement between Bentham & Hooker and Bremekamp. Their almost similar basic chromosome constitution ($x = 11/10, 11$) duly corroborates this. However, in palynological features, some degree of heterogeneity exists between and among them (Mathew & Philip 1983), such as many-colpate, 3-colporate and inaperturate in Psychotrieae; 3-colpate and 3-colporate in Anthospermeae; many colpate and 3-colporate in Spermacoceae. Most classifications have assigned the Galieae the highest position, except Verdcourt's (1958) who has placed this in a primitive position. But, chromosomally this is the most evolved tribe with striking degree of polyploid evolution on the derived basic constitution. This together with the evolved tricelled pollen grains evident in the Galieae accords strong support for its most advanced position.

Phylogeny and interfamily relationship

During the past two decades, as the molecular data have accumulated, there have been a tremendous increase in our understanding of the phylogeny of the Rubiaceae; and scores of phylogenetic reconstructions at different taxonomic levels have appeared. Of late, certain better pictures of the phylogeny of the family have forthcome as that of Bremer & Eriksson (2009), who have developed a time tree of rubiaceous phylogeny, dating at family, subfamily and tribe levels, and claimed strong support for the clades that correspond to their three subfamilies such as Cinchonoideae, Ixoroideae and Rubioideae and most of the 44 tribes. According to them, the reconstructions so far appeared could not resolve the intriguing question about the basalmost relationships of the Rubiaceae. Their phylogenetic tree shows a polytomy of four branches at the base of the family with Rubioideae, Cinchonoideae, Ixoroideae, Coptosapelteae and Luculieae. Concerning affinity of the Rubiaceae with other families, there is much discord. Bessey (1915) has kept this family in the order Rubiales along with a few families including Dipscaceae. Bentham & Hooker (1873) also kept this under the Rubiales, but along with only a few of Bessey's other families. Wagenitz (1959 cited in Bremekamp 1966) has included Rubiaceae in the order Gentianales along with Loganiaceae, Apocynaceae, Gentianaceae etc., considering the other families of Bessey's Rubiales under a different order, the Dipsacales. More recently, Bremekamp (1966) and Cronquist (1968) have proposed a separate order (Rubiales) for Rubiaceae. An overview of chromosome data of the Gentianales and Dipsacales shows that the $x = 11$ basic constitution is very frequent in all the gentianalean families, in contradistinction with those of the Dipsacales. In palynological features also the Gentianales and Dipsacales are much different, consistently euripalynous in the former and exclusively stenopalynous in the latter. In view of all these, Wagenitz's treatment of Rubiaceae under the Gentianales is appropriate. Bremekamp (1966) and Mathew & Philip (1986), based on pollen nuclear data, also favoured the gentianalian affinity. The most recent APG IV (2016) and Mabberley (2017) treatments also favour a gentianalian affinity. The proposed treatment of Rubiaceae as a monotypic order Rubiales as suggested by Bremekamp (1966) and Cronquist (1968) and in the APG IV (2016) is worth consideration. The most important points of resemblance between the Rubiaceae and its gentianalean allies are the presence of colleters and nuclear endosperm, while major differences are absence of intraxylary phloem and presence of inferior ovary. With respect to the latter two features, Rubiaceae resembles the families of the Dipsacales. But in Bremakamp's view, the character of inferior ovary is not so important a reason for exclusion of Rubiaceae from the gentianalean fold. Cronquist (1968) holds that the Rubiaceae form a connecting link between the Gentianales and Dipsacales. In this context, it is much more pertinent to consider the strong link and similarity between Rubiaceae and gentianalian families through the basic chromosome constitution of $x = 11$. However, there is notable difference between the two in the degree of occurrence of $x = 11$, which in the Rubiaceae is present with overwhelming profusion. Moreover, palynologically also the members of Rubiaceae are notably different from those of the gentianalean families as regards the degree of eurypalyny (Mathew & Philip 1983). In view of this, creation of a monotypic order Rubiales as proposed by Bremekamp (1966) and Cronquist (1968) also merits consideration and is worthwhile.

ACKNOWLEDGEMENT

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