III. CLADISTICS OF THE RUBIACEAE

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INTRODUCTION

Cladistics is a method by which the relationships among the taxa are quantified and a phylogenetic tree is erected. It is one of the two methods to formalise taxonomic methods and make the results repeatable accurately, the other being numerical taxonomy. Cladistic analysis attempts to reconstruct the branching pattern of phylogeny and hence to recognise clades or monophyletic groups from the distribution of derived characters. As a result a dendrogram similar to those of the numerical taxonomy is produced. The dendrogram of the numerical taxonomy represents the similarities and dissimilarities prevailing among various taxa and puts very less stress on the phylogeny. Cladogram on the other hand shows phylogeny of every taxon as well as their relationships.

In cladistics the organisms are marked entirely on the basis of recency of common discent i.e. on the basis of sequence of dichotomies in the inferred phylogeny. It provides a quantitative comparison of a large number of characters simultaneously and gives an efficient summary of character distribution, In this, phylogenetic interpretations which are vaguely defined and inconsistently used are quantified and formalised. As a result cladistics elevates systematics from intuitive taxonomy into a legitimate branch of science. The result thus obtained are accountable and consistent. Cladistics in the strict sense does not allow the existence of polyphyletic groups or an existing taxon to be ancestral to any other existing taxon. The cases of parallelism, convergence, divergence or hybridisation cannot properly be represented in a cladistic treatment. The polarity of the characters as well as the selection of outgroups are mostly left to the judgement of the taxonomists (Cronquist, 1986).

In most of the cladistic methods the number of theoretically possible cladograms is likely to be so large that it is necessary to use a computer to find out the most parsimonious one. There are diverse sorts of computer programmes all aimed at parsimony but likely to take different routes to approach it. Different methods of programming may lead to somewhat different results. Some of the programmes called for as many as 124 steps to produce the most parsimonious cladogram (Doyle and Donoghue, 1986).

Among the various methods available, 'Wagner network' and 'tree' are found to be more logical and can be constructed manually. Between these two, 'Wagner tree' is followed, because it is directional. Though expressing reservations on the indiscriminate usage of cladistics in deducing phylogeny, Cronquist suggests exercising a rational consideration in the selection of characters and advises preparing the cladogram manually on the basis of "Wagner's ground plan divergence method" (Wagner, 1980). In the present work 32 genera belonging to family Rubiaceae have been subjected to a cladistic treatment using characters selected from morphology, cytology, palynology and chemistry. "Wagner tree" is constructed by calculating the distances between taxa and this is superimposed on the "Wagner bull eye" diagram recommended for the ground plan divergence method.

METHODOLOGY

20 characters selected from morphology (4), cytology (1), palynology (3) and chemistry (12) have been used for the cladistic analysis. Polarity of the morphological characters is assessed following Cronquist (1968) and Takhtajan (1980) and cytological and palynological characters following Nair (1974) and Mathew and Philip (1983). The polarity in chemical characters are judged by the known biosynthetic pathways and correlation studies (Harborne et al., 1975).
The selected characters and their plesiomorphic and apomorphic states are presented in table IV. The distribution of characters in the taxa studied is presented in table V.
An advanced (apomorphic) state is given a score = 1 and a primitive (plesiomorphic) character is given 0 score. The "Wagner tree" is constructed based on the method of Jensen (1981).
Various steps for the construction of the tree are :

TABLE - IV

The characters used in cladistics and their Plesiomorphic and Apomorphic states

Sr No.	Characters	Plesiomorphic Score = 0	Apomorphic Score = 1
	Morphology		
1.	Habit	Shrub or tree	Herb
2.	Stipules	Present	Absent
3.	Inflorescence	Raceme or umbel	Head or cyme
4.	Fruit	Dehiscent	Indehiscent
	Cytology		
5.	Chromosome number	x = 11	x = More than 11 or less than 11
	Palynology		
6.	Pollen aperture	Colpate or tricolpate	3-Colporate 3-Porate Pororate Many-colpate Inaperturate
7.	Pollen nuclear number	Binucleate	Trinucleate and above.
8.	Exine character	Sculptured	Nonsculptured

Table - IV Contd.

Sr No.	Characters	Plesiomorphic Score = 0	Apomorphic Score = 1
	Chemistry		
9.	Flavones	Absent	Present
10.	Glycoflavones	Absent	Present
11.	Kaempferol	Absent	Present
12.	Quercetin	Present	Absent
13.	Methoxylated flavonols	Absent	Present
14.	Proanthocyanidins	Present	Absent
15.	Quinones	Absent	Present
16.	Iridoids	Absent	Present
17.	Alkaloids	Present	Absent
18.	Tannins	Present	Absent
19.	Syringic acid	Present	Absent
20.	Saponins	Absent	Present

TABLE - V

Distribution of various characters in the 32 genera studied

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	AD(I) Value
I SUBFAMILY - CINCHONOIDEAE																					
Adina	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	5
Cinchona	0	0	1	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	5
Rondeletia	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1	0	0	5
Stephegyne	0	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	1	1	0	0	6
Wendlandia	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	1	0	6
Gardenia	0	0	1	0	0	1	0	1	0	0	0	1	1	0	0	0	1	1	0	1	8
Hamelia	0	0	1	1	1	1	0	0	0	0	1	0	1	0	0	0	0	1	0	1	8
Pentas	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0	1	1	1	1	0	8
Ophiorrhiza	1	0	1	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	1	1	8
Catesbaea	0	0	1	1	1	1	0	0	0	0	1	1	0	1	0	0	0	1	0	1	9
Hymenodictyon	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	1	1	1	1	1	9
Mussaenda	0	0	1	1	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	1	9
Xeromphis	0	0	1	1	0	1	0	0	0	0	0	1	1	1	0	0	1	1	0	1	9
Anthocephalus	0	0	1	1	0	1	0	1	0	1	0	0	1	1	1	0	0	1	0	1	10
Dentella	1	0	0	1	1	1	1	0	0	0	1	0	1	1	0	0	1	1	0	0	10
Randia	0	0	1	1	0	1	0	0	0	0	0	1	1	1	0	1	1	1	0	1	10
Oldenlandia	1	0	1	0	1	1	1	0	0	0	1	0	0	1	1	1	1	1	0	1	12

TABLE - V Contd.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	AD(I) Value
II SUBFAMILY - COFFEOIDEAE																					
<u>Coffea</u>	0	0	1	1	0	1	0	0	0	0	0	1	0	1	0	0	1	1	0	0	7
<u>Meyna</u>	0	0	1	1	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	7
<u>Ixora</u>	0	0	1	1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	8
<u>Mitracarpum</u>	1	0	1	0	1	1	1	0	0	0	0	0	1	0	0	0	1	1	0	0	8
<u>Plectronia</u>	0	0	1	1	0	1	0	0	0	0	1	1	1	1	0	0	0	1	0	1	9
<u>Psychotria</u>	0	0	1	1	0	1	0	0	0	0	1	1	0	0	0	1	1	1	0	1	9
<u>Serissa</u>	0	0	1	1	0	0	1	0	0	1	0	1	0	1	0	0	1	1	0	1	9
<u>Borreria</u>	1	0	1	0	1	1	1	0	0	0	1	0	1	1	0	0	1	1	0	0	10
<u>Pavetta</u>	0	0	1	1	0	1	0	0	0	0	0	1	1	1	0	1	1	1	0	1	10
<u>Spermadictyon</u>	0	0	1	0	0	1	0	0	0	1	1	0	1	1	0	1	1	1	1	0	10
<u>Morinda</u>	0	0	1	1	0	1	0	0	0	0	1	1	1	1	1	1	1	1	0	0	11
<u>Knoxia</u>	1	0	1	1	1	1	1	0	0	0	0	1	0	1	0	1	1	1	1	1	13
<u>Spermacoce</u>	1	0	1	0	1	1	1	0	0	0	1	1	1	1	0	0	1	1	1	1	13
<u>Rubia</u>	1	1	1	1	0	1	1	1	0	0	0	1	0	1	1	1	1	1	0	1	14
<u>Galium</u>	1	1	1	1	1	1	1	1	0	0	0	1	0	1	1	1	1	1	1	1	16

1 - 20 Characters used - refer table IV

1. Manhattan distances, which are simply the sum overall n characters of the absolute differences between all the pairs of taxa, are calculated.

2. A hypothetical ancestor (ANC) having state 0 for all the characters is constructed and the Manhatten distances from ANC for each OTU are calculated; which form the AD (I) value (Total advanced characters).

3. OTU with lowest AD (I) value is taken and is connected to ANC.

4. OTU with next lowest AD (I) value is selected and is connected to the interval ANC-OTU-1 through hypothetical unit HTU1, which is a median of ANC, OTU 1 and OTU 2.

All the remaining OTUs with increasing order of AD (I) values are fitted subsequently into suitable intervals. This is done by calculating the distances between OTU and all the intervals of the tree using the formula.

$$d\{(X \text{ INT } (AB)\} = \tfrac{1}{2}\{d\,(X\ A)+d(X\ B)-d\,(AB)\}$$

Each OTU is fitted into the interval to which it is having minimum distance through proper HTUs. Once the Wagner tree is constructed it is superimposed on a Wagner "bull eye" chart. In a Wagner "bull eye" diagram a number of concentric semicircles having a common base point is prepared in which each semicircle represents a single evolutionary status.

These semicircles are given the numbers 1,2,3,4 etc., The total number of apomorphic characters corresponds to the total number of semicircles. The scores of a taxon (AD(I) values) gives the extent of advancement that the taxon attained and the corresponding semicircles in which it is to be placed. For example if the taxon 'A' has a total score of 15, then it is considered highly advanced and placed on the 15th semicircle.

The branching of the tree follows the Wagner tree construction method. A taxon occupies the semicircle corresponding to its AD(I) value. The most advanced taxon occupies the farthest semicircle and the taxon with lowest advanced characters occupies the nearest semicircle.

Characters used:

Morphological:

1. 0 = shrub or trees; 1 = herb.

   Herbs are considered advanced because the tree habit is always considered primitive and all the advanced taxa exhibit herbaceous habit.

2. 0 = stipules present; 1 = stipules absent.

   The stipules are extensions of the leaf base seen in a number of primitive angiosperm taxa. Therefore the absence of stipule is considered as an advanced character.

3. 0 = inflorescence is a raceme or umbel; 1 = inflorescence is a head or cyme.

Head is considered advanced among the racemose inflorescences because a single insect visitor can pollinate all the flowers within a head. Similarly cymose inflorescences also produce similar clusters of flowers and therefore considered advanced.

4. 0 = fruit is dehiscent; 1 = fruit is indehiscent.

Indehiscent fruits are seen in highly advanced families such as Asteraceae and Poaceae. Therefore, this condition is considered as advanced.

Cytological

5. 0 = chromosome number x = 11; 1 = chromosome number x = 12 or more, or 10 or less.

The basic chromosome constitution of x = 11 exhibited by members of Rubiaceae is considered to be the earliest constitution of the family, (Mathew and Philip, 1979a) and thus is considered primitive. Any deviation from the basic chromosome number is postulated to have originated secondarily by amphidiploidy (Mathew and Philip, 1979 b.), and hence considered advanced.

Palynological

6. 0 = pollengrains colpate or tricolpate; 1 = pollen grains 3 colporate, 3-porate, inaperturate, pororate or many-colpate.

The most primitive form of aperture type found in Rubiaceae are colpate and tricolpate condition. The tricolporate, 3-porate, pororate, and many-colpate forms are relatively advanced and the inaperturate forms are considered as highly advanced.

7. 0 = binucleate pollen nuclear number; 1 = pollen nuclear number trinucleate and above.

Binucleate pollen grains prevalent in the Rubiaceae is considered a primitive character. The trinucleate or multinucleate condition therefore is advanced.

8. 0 = pollen grains with sculptured exine; 1 = pollen grain with nonsculptured exine.

Pollen types with thick and heavily ornamented exine have been considered to be primitive, while those with thin and unornamented or lightly ornamented exine, advanced. (Wodehouse, 1931).

Chemical

9. 0 = flavone absent; 1 = flavone present.

Flavones are the most stable flavonoids found in Angiosperms. They are produced at the end of the biosynthetic pathways. Flavones are not located in any of the primitive families but are abundant in the advanced and most advanced plant taxa.

10. 0 = glycoflavone absent; 1 = glycoflavone present.

The presence of a flavone skeleton in glycoflavones keep them advanced over other flavonoids such as chalcones, flavonols etc., Though compared to the flavones, glycoflavones are slightly primitive, in this family where flavones are very rare glycoflavones are considered advanced.

11. 0 = kaempferol absent; 1 = kaempferol present.

12. 0 = quercetin present; 1 = quercetin absent.

13. 0 = methoxylated flavonol absent; 1 = methoxylated flavonol present.

The flavonols are associated with woody nature and therefore considered primitive. The tendency among flavonols is towards methoxylation of the hydroxy groups or reduction in the hydroxyl groups. Therefore, quercetin which is a pentahydroxy flavonol is considered primitive and kaempferol and methoxylated flavonols are considered evolved in this family.

14. 0 = proanthocyanidins present; 1 = proanthocyanidins absent.

18. 0 = tannins present; 1 = tannins absent.

Both proanthocyanidins and tannins are associated with woody nature. In the plants, they are protective in nature preventing the entry of microbes into the cells and organs. Since both these groups of compounds are polyphenolic, they are toxic to the living cell in larger amounts. This indicates that these are poor defensive mechanisms. Therefore, both these chemical characters are considered disadvantageous in the course of evolution and therefore primitive.

15. 0 = quinones absent; 1 = quinones present.

The significance of quinones in plants is still obscure. They may be considered advanced because of their antioxidant properties due to which they protect the cell organelles.

16. 0 = iridoids absent; 1 = iridoids present.

Iridoids form a defensive mechanism evolved late in plants. It is seen that these compounds replace tannins or proanthocyanidins in herbaceous Angiosperms. Being non-toxic to the protoplasm they can be stored in the living cells where they impart a bitter taste (preventing grazing) and being antimicrobial they protect the plants from pathogenic fungi or bacteria.

17. 0 = alkaloid present; 1 = alkaloid absent.

Alkaloids being bitter in taste offer protection against grazing but being alkaline in nature they interfere with the metabolic reactions of the cell. Therefore the alkaloids are seen stored predominantly in barks, root, fruit etc., away from sites of active biological reactions. The advanced group of plants tend to eliminate alkaloids and replace them with other mechanisms such as iridoids. Thus the presence of alkaloids may be considered a primitive condition.

19. 0 = syringic acid present; 1 = syringic acid absent.

Syringic acid is a component of angiosperm lignin. Evidently lignin is associated with woody nature and reduction in the amount of lignin is considered advanced. It is in this context the absence of syringic acid is considered as an advanced trait.

20. 0 = saponins absent; 1 = saponins present.

The significance of saponins in angiosperms is not yet clear. Many of the saponins are antimicrobial. It is also found that saponins are frequently seen in herbaceous plants. So the presence of saponins is considered advanced.

RESULTS AND DISCUSSION

The cladogram obtained by superimposing the 'Wagner tree' on a 'Wagner's bull eye' chart is presented in Fig.3. A separate dendrogram showing groupings of plants and their branch points is represented in Fig. 4. From the cladogram it is evident that *Galium* is the most advanced genus of the family (with AD (I) value:16) closely followed by *Rubia* (AD (I) value:14), *Knoxia* and *Spermacoce* (AD (I) values:13). Incidentally all these four genera belong to subfamily Coffeoideae. *Cinchona*, *Adina* and *Rondeletia*, having AD (I) value 5 each, are the most primitive genera of the Rubiaceae. All these three belong to the subfamily Cinchonoideae. In Cinchonoideae, *Oldenlandia* with AD (I) value 12 is the most advanced genus. The cladogram evidently pictures the Coffeoideae as the advanced of the two Subfamilies.

The dendrogram prepared clearly points to the groupings arrived at by using Wagner tree. Twelve groups are recognised. Many of them correspond to the acclaimed tribes of the family. Six groups belong to the Coffeoideae and the remaining six groups, to the Cinchonoideae. Each group is discussed in detail below.

The first group containing *Coffea*, *Serissa* and *Ixora* is similar to the tribe Ixoreae and Anthospermeae combined. *Coffea* is more closer to *Serissa* than to *Ixora*. *Serissa* is the advanced member of this group.

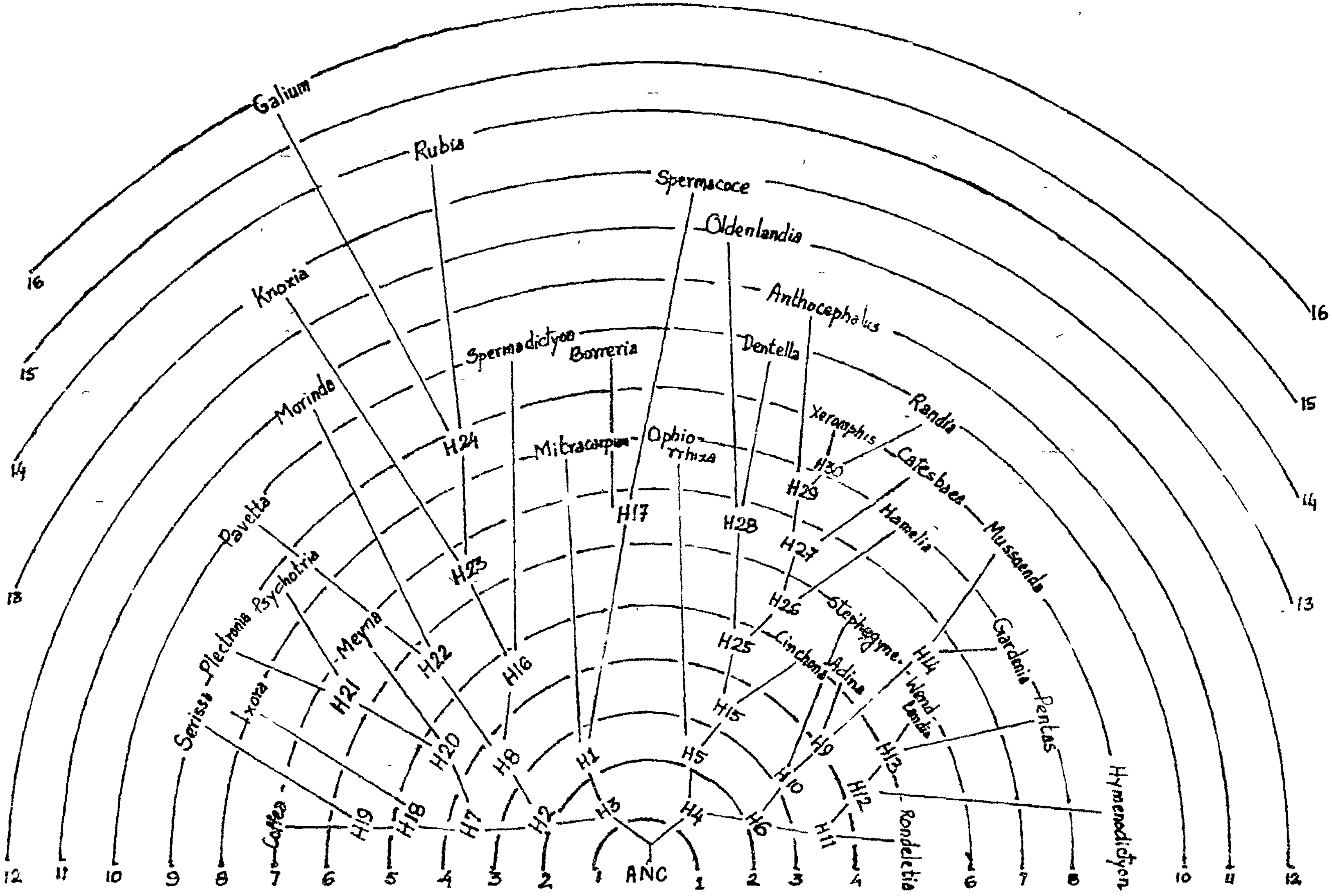
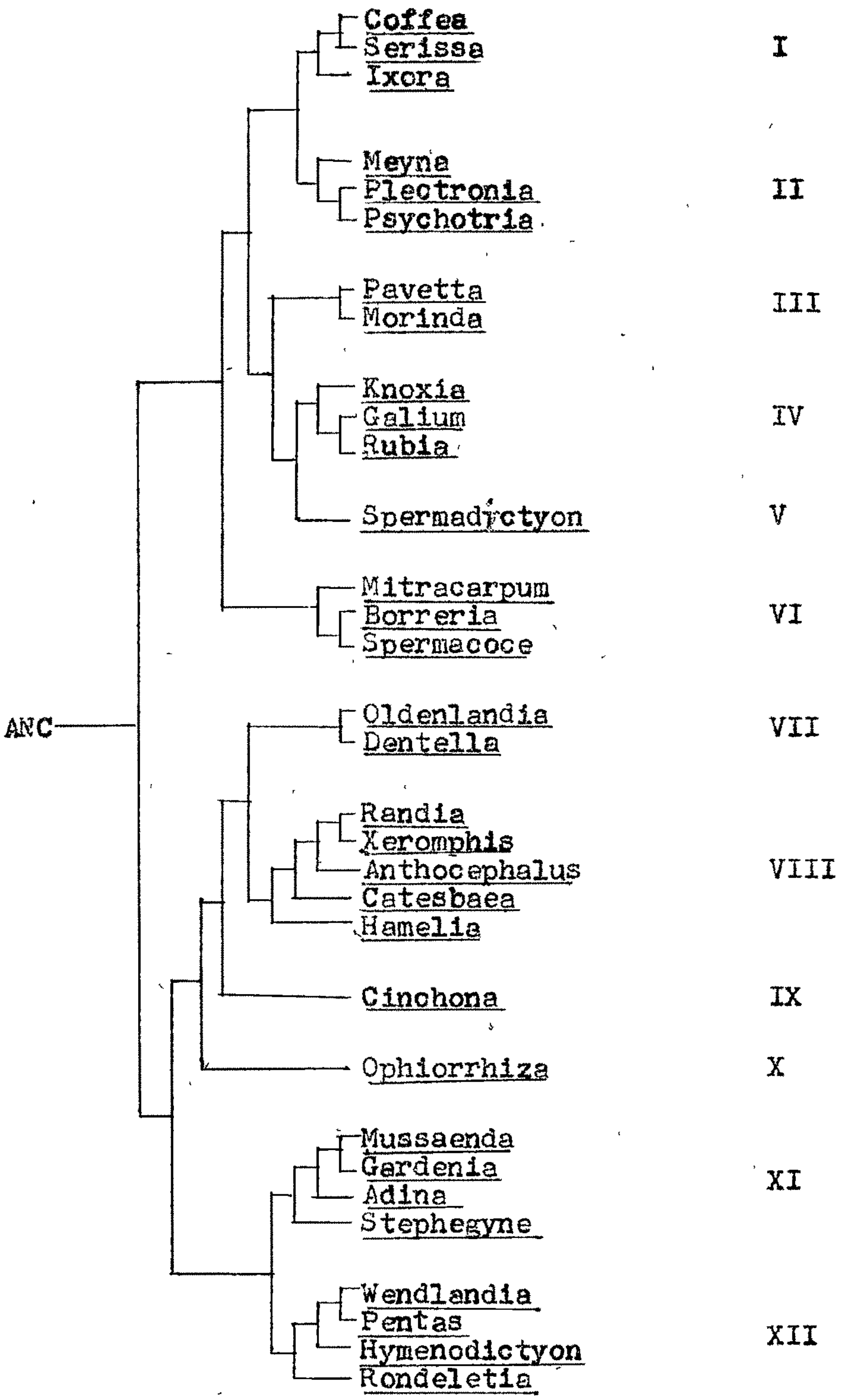


Fig 3. Cladogram depicting the interrelationships among the genera of the Rubiaceae

Fig. 4. Dendrogram showing groupings of plants and their branch points



The second group consists of Meyna, Plectronia and Psychotria which coincides with the tribes Vanguerieae and Psychotrieeae combined. Plectronia and Psychotria form an advanced subgroup.

The third group containing Pavetta and Morinda does not find any parallel in the existing classification. This group is advanced over the previous two groups.

The fourth group containing Knoxia, Galium and Rubia corresponds to the tribe Knoxieae and Galieae. The closeness of Rubia and Galium is noteworthy. This group forms the most advanced group of the family.

Spermadictyon forms an isolated group distantly connected to the group four.

Sixth group is the most natural collection of genera with Mitracarpum, Borreria and Spermacoce. This is the acclaimed Spermacoceae. Spermacoce is the most advanced genus in this group.

Group number seven is the first group of the subfamily Cinchonoideae. This contains Dentella and Oldenlandia which were grouped in the tribe Oldenlandieae. This group form the most advanced taxon within the Cinchonoideae.

The next group (eight) containing Randia, Xeromphis, Anthocephalus, Catesbaea and Hamelia is the largest taxon in the family which may be split up into two subgroups, the first one containing *Randia*, *Xeromphis* and *Anthocephalus* and the second group with the remaining two genera.

The species of *Cinchona* form the ninth group and *Ophiorrhiza* form the 10th group. *Ophiorrhiza* was grouped in the tribe Oldenlandieae from where it was shifted and elevated to a new tribe Ophiorrhizeae due to its peculiar characters (Refer the discussion on chemotaxonomy). The cladistic treatment places this genus separate from its allegedly related genera such as *Oldenlandia* and *Dentella*.

The 11th group also is a fairly large group containing *Mussaenda*, *Gardenia*, *Adina* and *Stephegyne*. This is one of the primitive groups.

The last group (12th) containing *Wendlandia*, *Pentas*, *Hymenodictyon* and *Rondeletia* correspond to the tribe Rondeletieae, Cinchoneae (in part) and Oldenlandieae (in part).

All these groups recognised may be accorded the status of tribes. The absence of any preconceived judgement or weightage and the automatic grouping resulted from the cladistic analysis keep these groups natural though some of the groupings appear heterogeneous to a classical taxonomist. A more clearer picture may emerge if more genera and more characters are taken into consideration. However the present analysis reinforces the circumscription of a number of acclaimed tribes.