BIOCHEMICAL AFFINITIES OF SOME SPECIES OF FICUS

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ABSTRACT

Polyphenolic constituents of eighteen Ficus species were studied as separated by paper chromatography. Their inter-relationship was examined on the basis of their paired affinity (PA) indices. A comparative relationship of each species in respect of PA values with others, are shown in polygonal graphs. Each species possess a distinct shape and area of these polygons. F. eriobstryoides, the only species native of Tropical Africa included, has distinctly smaller PA values thereby indicating its remote relationship with others.

About 25-30% of the polyphenolic constituents are common in all the species, probably an indication of common heritage. At least two phenolics are found to be common to all the species studied. PA indices between 30 and 60% are shown by a fairly large group of species in each case. Considering PA values above 60% or less than 30% as indicating more nearness or remoteness respectively, it is possible to show inter-relationship among them. The various species of the subgenus Urostigma clearly isolate themselves into one group. F. virens, F. elastica and F. drupaceae var. pubescens appear to be the connecting links between the species of the subgenus Urostigma and Ficus. Details of the relationship are discussed.

INTRODUCTION

The use of chromatographic data for a set of biochemical entities, is a valuable tool to explore species affinities. Such studies provide circumstantial evidence of supplementary nature and thus contribute to the understanding of phylogenetic relationship. Beta-Smith and Larner (1954) showed the distribution of leuco-anthocyanins in various plants and discussed at family level (Beta-Smith, 1956), the phylogenetic implications of its distribution. Acheson et al (1962) studied the distribution of anthocyanins in the flowers of the various species of the genus Papaver and their interspecific hybrids. Taylor (1964) conducted studies of the polyphenolic composition of the various species of the genus Coprosoma and concluded that "rapid quantitative analysis of polyphenols, without the identification of components could prove a great help in the study of hybridization, interogression species The inherent limitation of such relationship". biochemical information should not be overlooked. Any additional biochemical data substantiating the total biochemical background of the plants may Alston and Irwin alter their inter-relationship. (1961) and Matthews (1966) found only moderate or no variation in the free amino acids among Cassia and Tradescantia species respectively and pointed out that the pattern of variation of secondary substances offer a great potential in taxonomic work. The term "secondary constituents" was adequately defined by Erdtman (1956): "Taxonomically the most suitable substances seem usually to be those which are not involved in the primary metabolic process and which do not have any task to fulfil; in short natural products which have been regarded as unimportant and which are, in their biological environment, relatively stable by-products, often donated by the term secondary constituents". These substances offer a large pool of biochemical varia-The distribution pattern of these substances might be used for an understanding of phylogenetic relationship in biochemical terms. Any unqualified taxonomic conclusion from such a biochemical data had, however, been found somewhat difficult for want of suitable method to express the results in the form of quantitative relationship. One suggestion to this end, was given by Ellison, Alston and Turner (1962). These authors found their method quite suitable as applied to chromatographic data of the various species of the genus Bahia. In the present investigation, the methods enlisted by Ellison et al (1962) and subsequently followed by Matthews (1966), were applied to interprete the taxonomic relation of eighteen species of the genus Ficus on the basis of variations in polyphenolic constituents as separated on paper chromatograms.

MATERIAL AND METHODS

The following eighteen species of the genus Ficus were used in the study:

(1) Ficus religiosa L., (2) F. virens Ait., (3) F. drupaceae var. pubescens (Roth) Corner., (4) F. benghalensis L., (5) F. benghalensis var. krishnae (C. DC.) Corner., (6) F. mollis Vahl, (7) F. altissima Bl., (8) F. kurzii King, (9) F. benjamina L., (10) F. benjamina var. nuda (Miq.) Barrett., (11) F. elastica Roxb. ex Horman., (12) F. racemosa L., (13) F. pumila L., (14) F. hederacea Roxb., (15) F. heterophylla Linn. f., (16) F. auriculata Lour., (17) F. hispida Linn. f., (18) F. eriobotryoides Kunth & Bouche.

Species numbers 1 to 17 arc Asian and according to Corner's (1965) classification, nos. 1 to 12 are monoecious belonging to subgenus Urostigma, section Urostigma (nos. 1 & 2), section Conosycea (nos. 3 to 10), section Stilphnophyllum (no. 11) and subgenus Sycomorus (no. 12): nos. 13 to 17 are dioecious belonging to subgenus Ficus, section Rhizocladus (nos. 13 & 14), section Sycidium (no. 15), section Neomorphe (no. 16) and section Sycocarpus (no. 17). F. eriobotryoides is monoecious and is native of Tropical Africa (Hutchinson et al., 1954; Prain, 1917).

The plants of these species growing within the premises of the Indian Botanic Garden, Sibpore, Calcutta, were utilized for the present study conducted during July-October, 1966. The number of plants available for sampling varied from two to four. More than fifty, mature and healthy leaves were collected from each tree, washed thoroughly with final washings given with distilled water, cut into small bits, mixed up thoroughly and a sample of about one hundred grammes was picked out. The leaf material was homogenized with rectified alcohol in waring blender for five minutes. Two more washings with boiling 70% alcohol completed the process of extraction. Polyphenols with free orthohydroxyl group were separated by the addition of about 50 ml of saturated neutral lead acetate to the combined alcoholic extract. The remaining phenolics were precipitated by basifying the supernatant with 50% ammonia. Non-glycosidic and monoglycosidic material was extracted from these precipitates with ethyl acetate after decomposing it with 10 ml of 2 N sulphuric acid and 20 ml of saturated ammonium sulphate. The heavily glycosidic material left behind was hydrolysed under reflux after adding concentrated hydrochloric acid so as to make it 2N. The phenolics thus released were recovered in ethyl acetate. The original extract thus yielded four fractions of phenolics depending upon their nature of hydroxylation and degree of glycosidation. The method is the same as described by Taylor (1954). The ethyl acetate extracts were concentrated to small volumes and chromatographed (descending) on

Whatman's (no. 1) filter paper strip of 22×5 cm size using Partridge solvent (n-butanol: acetic acid? water; 4:1:5) in airtight cabinets. The paper strips were allowed about fifteen hours to attain equalibrium with the vapour phase of the same solvent before dipping their ends in solvent trough. The dried chromatograms were examined under U.V. light (Hanovia U. V. lamp). Each of the above four fractions were chromatographed at least three times for confirmation of Rf. values of the various spots. Minor variations in Rf. values of similarly flourescent spots in different chromatograms of the same fraction were settled by taking their mean values.

RESULTS

Under the influence of various agencies responsible for natural process of evolution, different types of phytochemical compounds are continuously formed as a result of changes in the genetic constitution. These secondary substances which are by-products of various metabolic processes are however, responsible for providing such constitution to a plant so as to allow a better performance under the existing conditions. These changes may be in the form of offering resistance to diseases, insects and pests or may have other regulatory functions. The disappearance of these substances provides clues to the study of natural process of evolution. In table I, a few more common phenolic substances are listed along with the Rf. values and colour under UV light. Only those spots are shown which are present in at least six or more of the eighteen taxa under consideration.

It is seen that there are at least two phenolics which are common to all these species. Both of them are heavily glycosidic phenols; one having free orthohydroxyl group, and the other devoid of this, appearing at Rf. 0.78 and 0.83 respectively. Both have the same blue colour under UV light. These two phenolics are not only present in all the Asian species under consideration but also in one from Tropical Africa (F. eriobotryoides). These two substances therefore, appear to remain most stable or may be treated as essential for the genus Ficus. Is it possible to take it as a generic character? Perhaps a study of more diverse forms in the genus will provide the answer.

The process of evolution results in changes in almost all directions. At least in the matter of these major phenolics, the changes may involve only in heavily glycosidic substances e.g. F. racemosa

TABLE I

Description of more common phenolics with their Rf. values and colour under UV light (+ & - indicate presence or absence)

F. religiosa	F. virens	F. drupaceae var. pubescens	F. benghalensis	F. benghalensis var. krishnae	F. mollis	F. altissima	F. kurzii	F. benjamina	F. benjamina var. nuda	F. elastica	F. racemosa	F. pumila	F. hederacea	F. heterophylla	F. auriculata	F. hispida	F. eriobotryoides	Rf.	Colour
A. + -	Pher + +	nolics + +	with	h free + +	-	_	ydroz +	kyl g + +	++	+	+	++	r mo + +	+	+	des) + +		0.75 0.55	Blue Blue
_	++	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	0.10 0.15	Brown Yellow
B. + + +	Phen + +	olics + + +	with - + +	free + + -	ortl	ho-hy + 	/drox + - +	cyl g: + + + +	roup: + + -	* (Ag	tlyco: + + + +	nes c + + + +	of ori	ginal + + + +	lly he + + +	eavily + + + +	y glyd + - -	osidic phenols 0.88 0.78 0.40 0.20	Yellow Blue Yellow Yellow Yellow
C. + +	Phen + +	olics +	pred+	cipita — —	ted + +	as si +	imple +	e lea — —	d sal	ts (A +	Aglyo + +	ones + 	or 1	mono +	glyc + +	oside + +	s) 	0.82 0.55	Blue Blue
D + + -	Phen - + + +	+ + + +	pred - + +	ripita + + - +	ted 2 + + + + - +	- - + +	nple - - + -	lead + + - + - +	salts - + +	(Agl + + + - +	ycon - + + -	es of + + + - +	origi + - + - +	inally + + + - +	y hea - + +	vily + + + + - +	glyco: + 	sidic phenols) 0.95 0.90 0.83 0.77 0.72	Brown Blue Blue Blue Yellow

and F. virens, F. drupaceae var. pubescens and F. hederacea. The changes may be in the direction of phenolics having free ortho-hydroxyl groups e.g. F. mollis and F. hispida; F. virens and F. auriculata; F. drupaceae var. pubescens and F. elastica; F. benghalensis and F. altissima; F. benghalensis var. krishanae and F. altissima; F. kurzii and F. benjamina var. nuda etc.

A true picture of the direction of different lines of natural phylogenetic evolution is however, provided by less common phytochemical substances. One or more unique substances may provide evidence for a particular position of a species in systematic classification. In the present study however, no phenolic was found as unique to any particular species. A less common substance is always shared by two or more species. Probably the same substances have appeared even though the lines of evolution had been different. It would be difficult therefore, to assess their relationship entirely on the basis of these less common spots. It is very difficult also to say that a preponderance of substances which

are common to a large group of species will represent characters more primitive in nature and vice versa.

It is thus evident that the evaluation of chromatographic data to peep into the process of natural evolution is quite difficult. In an attempt to have suitable comparison in the form of quantitative relationship, methods enlisted by Ellison et al (1962) were followed, in which any two species are compared on the basis of their percentage biochemical affinity. The total number of flourescent spots in all the four fractions were added so as to have a large number of substances for the purpose of comparison. In this study, number of total spots ranged between 17-20 per species. Paired affinity index between any two species was calculated as follows:

Paired affinity index:
$$\frac{\text{Spots in common for species A & B}}{\text{Total spots in A+B}} \times 100$$

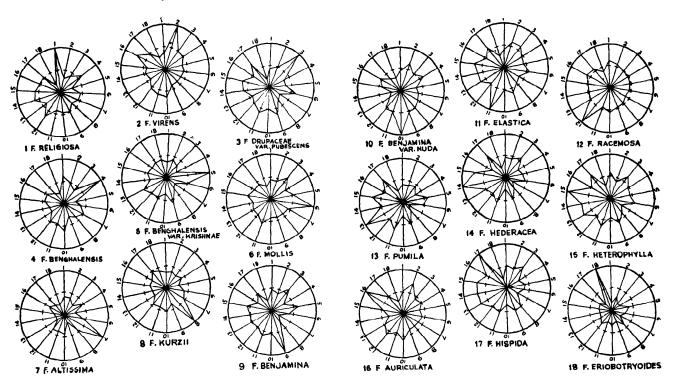
Polygonal graphs as suggested by Ellison et al (1962) for comparative relationship of a particular species in respect of its PA values with all others,

were drawn (Text fig. 1). Beginning with zero (centre), various PA values in respect of a particular species under consideration (PA value as 100, at the periphery of circle) are depicted.

The evaluation of biochemical affinity of the various species as evident from the polygonal graphs would indicate that each species has a distinct shape and area and none exactly match with each other.

Each of these species therefore, appears to be quite distinct. Excepting F. eriobotryoides and F. altissima, all the species possess about 25-30% of polyphenolic constituents in common. This is probably indicative of common heritage of the various species which subsequently have followed different pathways during the process of evolution.

This type of subjective evaluation of the bioche-



Text fig. 1: Polygonal graphs showing comparative relationship of each species on the basis of their paired affinity (PA) indices. Along each radius which represent one species in a circle, PA indices are plotted beginning with zero at the centre and 100 at the periphery.

mical data appear to give clear support of an independent identity of these eighteen species of Ficus as considered on the basis of morphological features. F. eriobotryoides possess distinctly smaller PA values indicating thereby its remote relationship with all others. This only species of Tropical Africa included in the present study, thus remains isolated.

F. altissima is still another species in this group, the PA values of which tend to isolate it from the rest. It's maximum affinity of fifty percent is only with F. benghalensis.

The differences in PA values of the various species with any one under consideration are grouped as indicated in table II. It would be seen once again that PA indices between 30 and 60 per cent are shared by a fairly large group of species in each case. Is it possible to consider PA values above 60% or less

than 30% as indicating more nearness or remoteness in terms of biochemical relationship? Though by all means of measurement this will be a sound basis for working out relationship, yet there are inherent objections and as such this can not be taken too strictly. However, under the present situation of limited information, the relationship among the various species is judged on basis of the above said consideration. Noteworthy features of this relationship are detailed below.

1. Unexpectedly, F. benghalensis bears a remote relationship with F. benghalensis var. krishnae. Paired affinity index between these two species is only 22%. It needs therefore, further clarification whether the later be called just a variety of the former species. The present data suggest very little connection between the two.

TABLE II

Groups of species with similar PA values. In column 3 to 6, a difference of $\pm 5\%$ of the value indicated is treated as negligible. All other species having PA value above 70% or below 30%, are included in column 2 and 7 respectively.

			Paired affinity index			
Name of the species	Above 70%	$65 \pm 5\%$	$55\pm5\%$	$45\pm5\%$	35 ± 5%	below 30%
1 .	2	3	4	5	6	7
1. F. religiosa	_	F. benghalensis F. racemosa	F. heterophylla F. auriculata F. hispida	F. drupaceae var. pubescens F. mollis F. allissima F. benjamina var. nuda F. elastica F. pumila F. hederacea F. benjamina	F. virens F. kurzii	F. benghalensis var. krishnae F. eriobotryoide
2. F. virens	F. auriculata	F. benjamina var. nuda F. elustica	F. drupaceae var. pubescens F. benghalensis F. racemosa	F. benghalensis var. krishnae F. mollis F. altissima F. kurzii F. benjamina F. pumila F. hederacea F. heterophylla F. hispida	F. religiosa	F. eriobotryoides
3. F. drupaceae var. pubescens		F. mollis F. benjamina F. elastica F. heterophylla F. hispida	F. virens F. racemosa F. hederacea	F. religiosa F. benjamina var. nuda F. pumila F. auriculata	F. benghalensis F. benghalensis var. krishnae F. kurzii F., altissima	F. eriobotryoides
4. F. benghalensis	_	F. religiosa F. benjamina var. nuda	F. virens F. altissima F. racemosa	F. kurzii F. elastica F. heterophylla F. auriculata	F. drupaceae var. pubescens F. mollis F. benjamina F. pumila F. hederacea F. hispida	F. benghalensis var. krishnae F. eriobotryoides
5. F. benghalensis var. krishnae	_	F. benjamina	F. mollis F. pumila	F. virens F. elastica F. racemosa F. hederacea F. heterophylla F. auriculata F. hispida F. eriobotryoides	F. drupacea var. pubescens F. kurzii F. bejamina var. nuda	F. religiosa F. benghalensis F. altissima
6. F. mollis		F. drupaceae var. pubescens	F. benghalensis var. krishnae F. elastica F. heterophylla	F. religiosa F. virens F. altissima F. kurzii F. benjamina var. nuda F. racemosa F. pumila F. hederacea F. auriculata F. hispida	F. benghalensis	F. eriobotryoides
7. F. altissima			F. benghalensis	F. religiosa F. virens F. mollis F. kurzii F. benjamina var. nuda	F. hispida F. drupaceae var. pubescens	F. benghalensis vax. krishnae F. benjamina F. pumila F. hederacea F. heterophylla

TABLE II-Contd.

			TABLE II-Conto	i.		
1	2	3	4	5	6	7
				F. elastica F. racemosa F. auriculata		F. eriobotryoides
8. F. kurzii	_		F. benjamina var. nuda F. hispida	F. virens F. benghalensis F. mollis F. altissima F. elastica F. eriobotryoides	F. religiosa F. drupaceae var. pubescens F. benghalensis var. krishnae F. benjamina F. pumila F. hederacea F. heterophylla F. auriculata	
9. F. benjamina	-	F. drupaceae var. pubescens F. benghalensis var. krishnae F. elastica	F. benjamina var. nuda F. pumila F. hederacea F. heterophylla F. hispida	F. religiosa F. virens F. mollis F. racemosa	F. benghalensis F. kurzii F. auriculata	F. altissima F. eriobotryoides
0. F. benjamina var. nuda		F. virens F. benghalensis	F. kurzii F. benjamina F. elastica F. racemosa F. auriculata	F. religiosa F. drupaceae var. pubescens F. mollis F. altissima F. heterophylla	F. benghalensis var. krishnae F. hispida F. eriobotryoides	F. pumila F. hederacea
1. F. elastica	_	F. virens F. drupaceae var. pubescens F. benjamina F. pumila F. hederacea F. heterophylla	F. mollis F. benjamina var. nuda F. hispida	F. religiosa F. benghalensis F. benghalensis var. krishnae F. altissima F. kurzii F. racemosa F. auriculata	F. eriobotryoides	<u>-</u>
2. F. racemosa		F. religiosa	F. virens F. drupaceae var. pubescens F. benghalensis F. benjamina var. nuda F. pumila F. heterophylla F. auriculata F. hispida	F. benghalensis var. krishnae F. mollis F. altissima F. kurzii F. benjamina F. elastica E. hederacea	F. eriobotryoides	
3. F. pumila	F. heterophylla	F. elastica F. hispida	F. benghalensis var. krishnae F. benjamina F. racemosa F. hederacea	F. religiosa F. virens F. drupaceae var. pubescens F. mollis F. auriculata	F. benghalensis F. kurzii	F. altissima F. benjamina var. nuda F. eriobotryoides
t. F. hederacea	F. heterophylla	F. elastica F. hispida	F. drupaceae var. pubescens F. benjamina F. pumila	F. religiosa F. virens F. benghalensis var. krishnae F. mollis F. racemosa	F. benghalensis F. kurzii F. auriculata	F. altissima F. benjamina var. nuda F. eriobotryoides
5. F. heterophylla	F. pumila F. hederacea	F. drupaceae var. pubescens F. hispida F. elastica	F. religiosa F. mollis F. benjamina F. racemosa F. auriculata	F. virens F. benghalensis var. krishnae F. benjamina var. nuda	F. kurzii	F. altissima F. eriobotryoides
6. F. auriculate	F. virens		F. religiosa F. benjamina var. nuda F. racemosa F. heterophylla F. hispida	F. drupaceae var. pubescens F. benghalensis F. benghalensis var. krishnae F. mollis	F. kurzii F. benjamina F. hederacea	F. eriobotryoides

TABLE II-Contd.

1	2	3	4	5	6	7
		-		F. altissima F. elastica F. pumila		
17. F. hispida		F. drupaceae var. pubescens F. pumila F. hederacea F. heterophylla	F. religiosa F. kurzii F. benjamina F. elastica F. racemosa F. hispida	F. virens F. benghalensis var. krishnae F. mollis	F. benghalensis F. altissima F. benjamina var. nuda	F. eriobotryoides
18. F. eriobotryoides	_			F. benghalensis var. krishnae F. kurzii	F. benjamina var. nuda F. racemosa	F. virens F. mollis F. religiosa F. drupaceae var. pubescens F. benghalensis F. altissima F. elastica F. pumila F. hederacea F. hispida F. heterophylla F. auriculata

- 2. F. virens have 75% of polyphenolic constituents in common with F. auriculata indicating a close relationship, though morphologically, the former is monoecious and the later dioecious.
- 3. F. virens is also closely related to an extent of 67% and 62% with F. benjamina var. nuda and F. elastica respectively.
- 4. F. benjamina var. nuda is related to the extent of 68% with F. benghalensis.
- 5. F. benghalensis is sufficiently close to F. religiosa (65%).
- 6. F. religiosa is fairly close to F. racemosa (55%).
- 7. F. elastica, apart from its close association with F. virens as stated above, is also near to F. benjamina (62%), F. drupaceae var. pubescens (65%), F. heterophylla (70%), F. hederacea and F. pumila (65%).
- 8. F. benjamina bears close association with F. benghalensis var. krishnae and F. drupaceae var. pubescens (62% & 65% respectively).
- 9. F. drupaceae var. pubescens is closer to F. mollis.
- 10. Like F. elastica, F. drupaceae var. pubescens appear to be closer to two dioecious species of subgenus Ficus. They are F. hispida (68%) and F. heterophylla (65%).
- 11. Among the dioecious species themselves, F. hispida is similar to F. pumila, F. hederacea, F. hterophylla by 68%, 65% and 70% respectively; F. pumila to F. heterophylla by 75% and F. hederacea to F. heterophylla by 70%.

12. On the basis of working relationship considered here, it is not possible to fix up any connection of the two species, F. altissima and F. kurzii. Considering however, paired affinity values between 50% and 60% as evidence of comparatively closer relationship, one may conclude that F. kurzii is more near to F. benjamina var. nuda (52%) and F. altissima to F. benghalensis (50%).

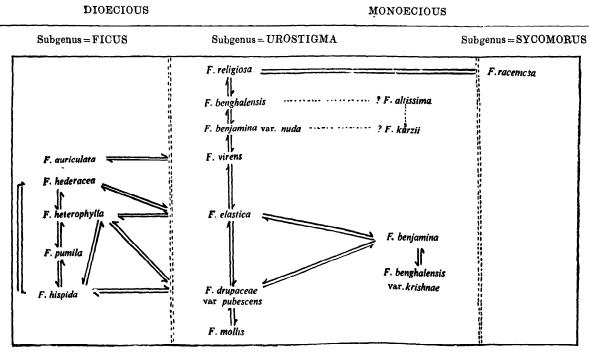
DISCUSSION

Chromatographic data of the polyphenolic complex of seventeen taxa of the genus Ficus distributed in Asian countries and one from the Tropical Africa indicate a clear distinction among all of them. Each one appear to have distinct identity. Comparative evaluation on the basis of polygonal graphs drawn with the values of their paired affinity indices, conspicuously isolate F. eriobotryoides, a Tropical African species from the rest indicating a distant relation, if any. The only Asian species which has a tendency to isolate itself is F. altissima.

Considering PA values above 60% as indicative of somewhat closer relationship and those below 30% as its remoteness, the various species (excluding F. eriobotryoides, which do not have any close relationship with the rest) studied appear to bear an inter-relationship among themselves as indicated below (Text fig. 2).

Admittedly, the number of species studied are too small when compared to the total morphologically described species (approx. 500). But for the purpose of comparative phytochemical study such a group is perhaps quite convenient.

The various species of the subgenus *Urostigma* clearly isolate themselves into one group, thus confirming to their morphological classification. The



Text fig. 2: Diagram depicting phytochemical relationship among various species of genus Ficus.

discripencies however, arise at the level of Sections. F. religiosa and F. virens are both included in the section Urostigma whereas phytochemically F. religiosa appear to be more near to F. benghalensis of section Conosycea than to F. virens. Similarly, F. benghalensis and F. benghalensis var. krishnae are put together systematically in section Conosycea series Drupaceae and subsection Indica, have very little polyphenolic constituents in common. It is worthwhile re-examining the question as to whether the later be treated as a variety of the former or be given a distinct specific status. Similar is the case of F. benjamina and F. benjamina var. nuda.

The three species of subgenus Urostigma viz. F. virens (section Urostigma, series Caulobotryeae), F. elastica (section Stilphnophyllum) and F. drupaceae var. pubescens (section Conosyeae, series and sub-series Drupaceae) are the only three species in the present group which show closer ties with the four dioecious species of the sub-genus Ficus. Whereas the former is near only to one species (i.e. F. auriculata of section Neomorphe) the latter two possess inter-relationship with the remaining four viz., F. hederaceae (section Rhizocladus series Dis-

tichae), F. heterophylla (section Sycidium, subsection Varinga), F. hispida (section Sycocarpus, subseries Hispidae) and F. pumila (section Rhizocladus, series Plagiostigmaticae) as indicated. The present study provides some evidence therefore, of inter-relationship between dioecious and monoecious groups and it appears that F. elastica and F. drupaceae var. pubescens along with F. virens are the connecting.

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