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TAXONOMY

Taxonomic reconsideration of *Calamus rivalis* Thw. ex Trim. and *C. metzianus* Schlecht (Arecaceae) through morphometric and molecular analyses

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ABSTRACT. Molecular and morphometric analyses of different Indian and Sri Lankan populations of two rattan species (*Calamus rivalis* and *C. metzianus*) were carried out using Random Amplified Polymorphic DNA (RAPD) markers and morphological characters. Multivariate analysis of RAPD and morphological data failed to separate these populations into two distinct species. RAPDs generated a total of 117 markers with 10 decamer primers, of which 95 percent were polymorphic. The percentage of polymorphic loci between populations varied from 26.5 to 68.38, and genetic distance between populations ranged from 0.05 to 0.28. The genetic variation within populations was greater than the variation among populations. Both the RAPD and morphometric data suggest the existence of a single species, and hence *C. rivalis* can be merged to *C. metzianus*.

Keywords: *Calamus*; Dendrogram; Genetic diversity; RAPD.

INTRODUCTION

Rattans are spiny climbing palms belonging to the subfamily Calamoideae of the Arecaceae (Uhl and Dransfield, 1987). They comprise about 600 species in 13 genera distributed in equatorial Africa, South Asia, Southern China, the Malay Archipelago, Australia and the Western Pacific. Rattans form one of the major non-wood forest products in international trading. Approximately 700 million people trade or use rattan for different purposes worldwide mainly for furniture and cottage industries. The global trade and subsistence value of rattan and its products is estimated at over US$ 7,000 million per annum (Pabuayan, 2000). Due to overexploitation, habitat degradation and low regeneration capacity, the rattan resources of the world are under serious threat. It is estimated that around 117 species of rattans are treated as threatened to some degree (Walter and Gillet, 1998).

In India, rattans comprise about 60 species in four genera—viz. *Calamus*, *Daemonorops*, *Korthalsia*, and *Plectocomia*—distributed in the Western Ghats, North Eastern states, and the Andaman and Nicobar Islands. *Calamus rivalis* and *C. metzianus* are two slender diameter rattans found in the Western Ghats and Sri Lanka. *Calamus metzianus* was first described by Schlecht (cf. Beccari, 1908) based on specimens collected by Rev. Metz from the Canara district of Karnataka, India. It was later discovered in Nilambur of Kerala, India (Renuka and Bhat, 1987). *Calamus rivalis* was originally described from Sri Lanka by Thwaites and was validly published by Trimen (Beccari, 1908). This species was also distributed in Ashramam (Kollam) and Chertala (Alapuzha) of India. Beccari (1908) suggested that *C. metzianus* represented the continental form of *C. rivalis* and this species could be distinguished from *C. metzianus* by a larger fruit size and distinctly channeled fruit scales.

The purposes of the present study are to examine the patterns of phenetic structure and levels of genetic variation within and among populations of these two species using random amplified polymorphic DNA (RAPD) markers and morphological characters. The RAPD protocol is relatively quick, easy to perform, and requires no sequence information prior to analysis and only a nanogram quantities of DNA (Williams et al., 1990).

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MATERIALS AND METHODS

Morphological characters

Two populations of *C. rivalis* from the Western Ghats of India and three from Sri Lanka and two populations of *C. metzianus* from the Western Ghats were included in this study (Table 1). Herbarium specimens were collected from all these populations. The voucher specimens were deposited in the KFRI herbarium. The details of OTUs (Operational Taxonomic Units), included for the morphometric analysis, are available on request. The characters were chosen on the basis of differences among taxa in the vegetative and reproductive parts. Only the mature plants were considered for the study, and a total of 32 qualitative and quantitative characters were selected. Qualitative characters were scored as 0 for absence and 1 for presence. Multiple herbarium sheets from a single collection were grouped into a single OTU; 107 such OTUs were used for the analysis.

RAPD analysis

Sample collection. Juvenile leaves were collected from two populations in Honaver and Nilambur for *C. metzianus* and from five populations in Chertala, Ashramam of India and from Watareke, Yagirala, and Matugama in Sri Lanka for *C. rivalis*. A total of 60 individual samples were selected for the analysis (Table 1).

DNA Extraction. The leaf specimens were collected in plastic bags and chilled at -20°C until DNA was extracted. For Sri Lankan samples, dried materials were used for DNA isolation. Total DNAs were extracted from 1 g of the leaf tissues using the modified CTAB protocol (Doyle and Doyle, 1990).

RAPD Reaction. PCR-RAPD analysis was carried out according to the Williams et al. (1990) protocol using ten primers, viz. OPAU02, OPA04, OPA18, OPAW07, OPAW09, OPAW10, OPAW20, OPE02, OPE18 and OPB15 (Operon Technologies, Alameda, CA), which were selected out of the 65 primers screened, based on the repeatability of their DNA band profiles. Amplifications were performed on a PTC-100 Thermal cycler (MJ Research Inc., USA) in 25 µL reaction mixtures containing 50-100 ng of template DNA, 200 µM each of dATP, dTTP, dCTP and dGTP, 3 units of Taq DNA polymerase, 1 µL (20 pm) of each primer, and 5 µL Taq buffer with 1.5 mM $MgCl_2$ (Genei, Bangalore, India). The amplification was performed with 45 cycles, each of 60 s denaturation (94°C), 60 s annealing (36°C), and 120 s extension (72°C). The last cycle was followed by incubation for 10 min at 72°C.

Electrophoresis of the amplified products. The RAPD products were electrophoresed on 1.5% agarose gel (Sigma, USA) in TBE buffer (40 mM Tris-borate, 1 mM EDTA, pH 8.0). The gel, after the completion of electrophoresis, was stained with ethidium bromide, and the bands were compared with DNA markers [100-bp DNA ladder and Low range DNA ruler (Genei)]. The gels were documented using a Kodak Digital Science Electrophoresis Documentation and Analysis System 120 (Kodak, USA).

Data analysis

Morphological characters. Cluster analysis and ordination were performed using NTSYS (Rolf, 2000). For cluster analysis, the standardized data were used to compute a distance matrix based on Average taxonomic distances, Manhattan distances, and Euclidian distances before being subjected to the unweighted pair-group method with an arithmetic averages (UPGMA) clustering algorithm. To test the consistency between the resulting phenogram and the original data, co-phenetic correlation coefficients were calculated for each phenogram and data matrix pair. The phenogram with the highest co-phenetic correlation coefficient (r) was reported here.

Table 1. Details of selected populations of *C. metzianus* and *C. rivalis* and comparison of various genetic diversity measures.

Population	Country	Latitude (ºN)	Longitude (ºL)	No of plants		h	npl	ppl
				RAPD	Morphology			
C. metzianus								
Honaver, Western Ghats	India	14º08’	74º30’	10	15	0.1637	47	40.17%
Nilambur, Western Ghats	India	11º17’	76º28’	10	20	0.2458	80	68.38%
C. rivalis								
Ashramam, Western Ghats	India	08º53’	76º35’	10	18	0.2086	66	56.41%
Chertala, Western Ghats	India	09º42’	76º19’	10	15	0.2326	69	58.97%
Watareke	Sri Lanka	06º 51’	80º03’	10	13	0.1633	54	46.15%
Yagirala	Sri Lanka	06º28’	80º11’	5	13	0.1085	31	26.50%
Matugama	Sri Lanka	06º31’	80º07’	5	13	0.1331	38	32.48%
Mean						0.1793	55	47.00%

h = Nei’s (1973) gene diversity; npl = Number of polymorphic loci; ppl = Percentage of polymorphic loci.

For ordination analyses, we carried out principal coordinate analysis (PCOA) for a mix of qualitative and quantitative data and principal component analysis (PCA) for quantitative data. Additionally, from the standardized fruit data, average Euclidean distance was calculated for each population pair to test the correlation between the average Euclidean distance and the genetic distance using a mantel test.

RAPD analysis. RAPD products were scored for presence (1) and absence (0) of bands. The data matrices were analysed using the Popgene, Version 1.31 package, and a pairwise comparison of populations was made (Yeh et al., 1999). The genetic diversity parameters within populations, viz. number of polymorphic loci and gene diversity, were determined. Genetic differentiation between the analysed populations was calculated according to Nei (1973).

Genetic distances (Nei, 1978) between all populations were obtained from Popgene, Version 1.31, and the resulting distance matrix was then used to construct an unrooted phenetic tree of the different populations using the Fitch Program of PHYLIP, Version 3.5 (Felsenstein, 1993). To evaluate the correlation between genetic distance and geographic distance, the product moment correlation coefficients were calculated between the genetic and geographic distance matrices, and significance levels of the correlation between these matrices were estimated by a mantel test using TFPGA software, Version 1.3. Analysis of molecular variation (AMOVA; Excoffier et al., 1992) was used to estimate variance components and to test the significance of the partitioning of RAPD variation among regions and among and within populations.

Cluster analysis and principal coordinate analysis were carried out using the NTSYS software package, Version 2.1 (Rolf, 2000). Genetic similarities based on Jaccard's coefficient (Jaccard, 1908) were calculated among pairs using the SIMQUAL option and ordered in a similarity matrix. The similarity matrix was run on sequential, agglomerative, hierarchical, and nested clustering (SAHN) (Sneath and Sokal, 1973) using UPGMA (unweighted pair group method with arithmetic average). Cophenetic correlation was calculated to measure goodness of fit. Principal co-ordinate analysis (PCO) was performed using the following modules of the NTSYS program: STAND, SIMINT, DECENTER and EIGEN to identify the number of groups based on eigen vectors. Three-dimensional ordination provided an additional representation of genetic relationships among the individuals of populations.

RESULTS

Morphological characters

The phenogram constructed using Manhattan distances showed the least distortion with a co-phenetic correlation coefficient, r = 0.8713. In euclidian distances, r = 0.86829, and in average taxonomic distances, r = 0.8682 (data not shown). The OTUs fell into three major clusters. The first and second clusters were composed of a complex series of closely nested clusters made up of a majority of the plants of the Western Ghats (Honaver, Nilambur, Chertala and Ashramam) and all of the OTUs from Matugama of Sri Lanka. The third cluster included all remaining Sri Lankan OTUs and some plants from Chertala. The first cluster (Hon 1 to Nil 11) consisted mainly of OTUs from Chertala and Ashramam with which the OTUs from Honaver and Nilambur overlapped. The second cluster (Nil 10 to Hon 13) includes the plants from Nilambur, Ashramam, Honaver, Chertala, and Matugama. The OTUs from Nilambur, Honaver and Chertala were found to be scattered among different clusters. The majority of OTUs from Ashramam were clustered together with OTUs from Nilambur. Among Sri Lankan specimens, OTUs from Matugama formed a distinct group linked with some OTUs of Chertala, Honaver, Watareke, and Yagirala.. The remaining OTUs, from Watareke and Yagirala, are scattered together and intermingled with OTUs from Chertala.

In ordination, the first principal components account for 38.58% of the total variation, with leaf sheath spine length, number of male rachilla/partial inflorescence, number of female rachilla/partial inflorescence, fruit apical beak length, number of fruits per rachilla, inflorescence caudex length, and fruit diameter having the highest loadings (Table 2). The second principal component explained 22.25% of the total variation, with stem diameter, nature of leafsheath spine and seed length having the highest loadings. The third principal component explained 9.17% of the total variation, with leaflet length, inflorescence, and caudex length having the highest loadings.

In Principal Coordinate Analysis, the first three principal co-ordinates explained a total of 69% of the total variation (Table 3) and failed to disclose any major gap in the pattern of morphological variation (Figure 1), clearly indicating the lack of distinct phenetic structure. However, OTUs from geographic regions do tend to fall together in a given proportion of the three dimensional factor space, as happens in cluster analysis.

RAPD analysis

Primer utility. Sixty-five primers from Kit OPA, OPB, OPE, OPAU and OPAW (Operon Technology, USA) were initially screened. Out of these, ten were chosen for further analysis based on the number and reproducibility of amplified products. A total of 117 markers were obtained, with molecular sizes ranging from 100 bp to 2,500 bp. Polymorphism was very pronounced, with 95% polymorphic markers across all primers.

The percentage of polymorphic loci (ppl) varied from 26.5 in the Matugama population to 68.38 in the Chertala population. The Chertala population had highest gene diversity index (h = 0.25) and the Matugama population of Sri Lanka the lowest (h = 0.11) (Table 1).

Table 2. Eigen vector coefficient for principal component analysis. Character loading values are for the first three Principal Component (PC) axes.

No.	Characters	PC 1	PC 2	PC 3
1.	Leafsheath spine length	0.8674	0.2061	0.1302
2.	Female rachillae length	0.8515	-0.0476	0.1849
3.	Inflorescence caudex length	0.8120	-0.1423	0.3063
4.	Fruit diameter	0.7826	0.0027	-0.2615
5.	Number of fruits per rachillae	0.7012	-0.0399	-0.0737
6.	Number of male rachilla	0.6809	-0.0919	-0.3409
7.	Number of female	0.6809	0.1761	-0.2767
8.	Internodal length	0.5943	0.4375	-0.0841
9.	Rachillae prophyll length	0.5345	0.1184	0.1657
10.	Leaflet length	0.5021	0.1985	0.3648
11.	Stem diameter	0.3242	0.5600	0.0715
12.	Seed length	0.1669	0.5111	-0.2912
13.	Partial Inflorescence length	-0.1342	0.3417	-0.6384
14.	Number of vertical rows of scales	-0.4868	0.3799	-0.3524
	Eigen values	9.147	3.920	2.935
	Percentages	28.584	12.251	9.174
	Cumulative percentage	28.584	40.835	50.010

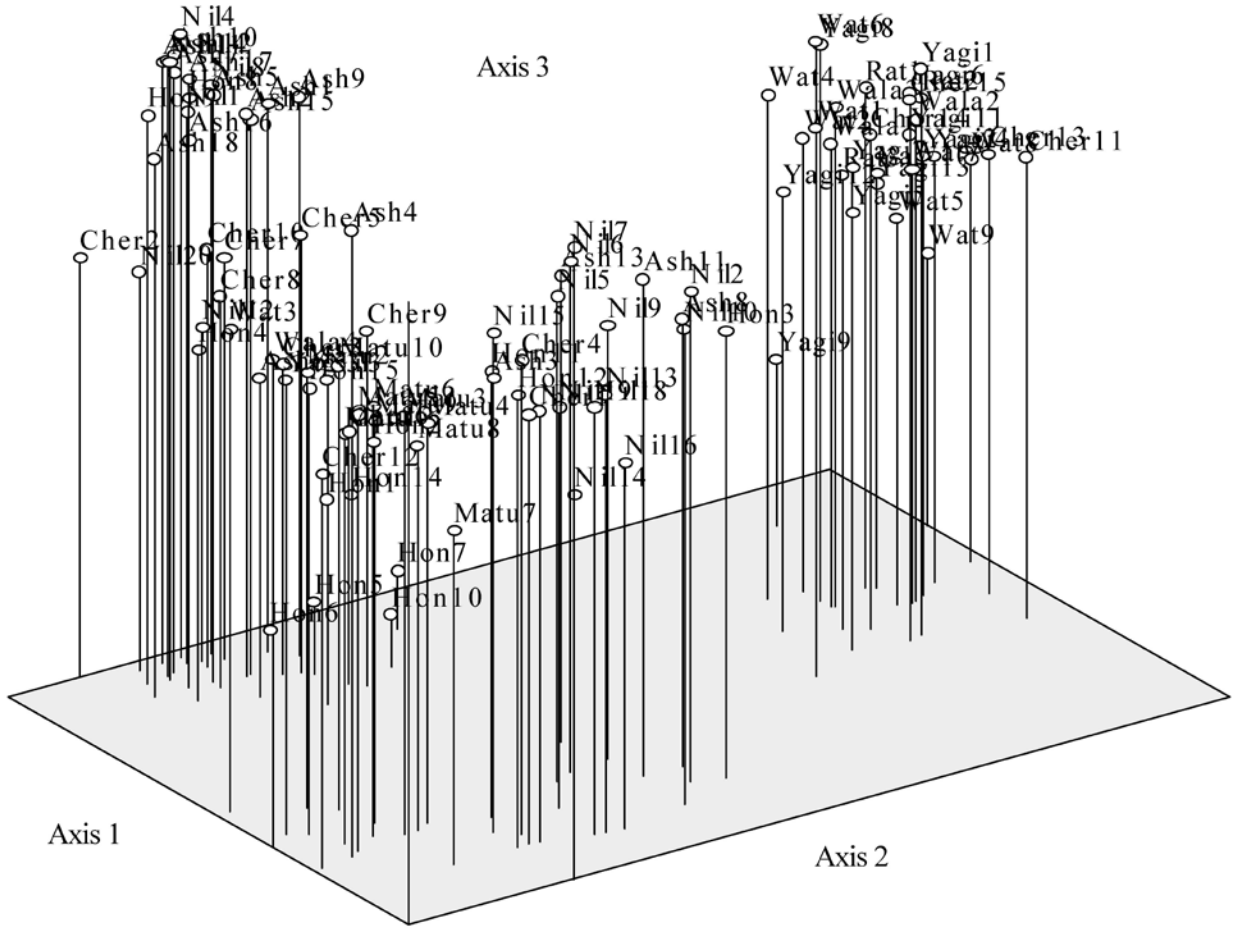

Figure 1. Principal coordinate analysis (PcoA) of 32 morphological characters. (H: Honaver; N: Nilambur; A: Ashramam; C: Chertala; W: Watareke; Y: Yagirala; M: Matugama).

The estimated average genetic distances between populations ranged from 0.05 (between Yagirala and Watareke) to 0.28 (between Yagirala and Honaver). Partitioning of variation within and between populations using an analysis of molecular variance (AMOVA) showed that 65.74% of the genetic variability existed as variation between populations (P<0.001; Table 4). When all the populations of the Western Ghats were treated as one region and the Sri Lankan as another region, the AMOVA result showed that the percentages of variation attributable to the differences between regions, among populations within regions, and among individuals within populations were 18.77% (P<0.001), 21.36%, (P<0.001) and 59.87% (P<0.009; Table 4).

Mantel test. The mantel test did not reveal any correlation between fruit morphological characters and genetic distance (r = 0.0156; p = 0.4800) or between the presence/absence of fruit scales and genetic distance (r = 0.0974; p = 0.6050). Geographical and genetic distances were significantly correlated (r = 0.7327; p = 0.0090).

The unrooted phenetic tree (Figure 2) constructed based on the genetic distance showed a clear distinction between the Indian and Sri Lankan populations. Two clusters were observed within the Indian populations (between Honaver/ Chertala and between Nilambur/Ashramam). Among the Sri Lankan populations, Yagirala and Watareke formed a single cluster, which in turn was linked to the Matugama population.

Cluster analysis. The UPGMA dendrogram, constructed based on Jaccard's similarity coefficients, depicted the genetic clustering of all the sixty genotypes analysed (data not shown). The cophenetic correlation coefficient between the dendrogram and the original distance matrix was 0.84. Three major clusters were observed in the dendrogram. The first cluster consisted of the individuals Honaver and Chertala with a similarity of 0.57. The second cluster associated the individuals of Ashramam and Nilambur with a similarity of 0.56. Two individuals of Chertala are clustered with individuals of Ashramam and Nilambur. The third cluster consists of entire Sri Lankan populations, which share a similarity of 0.61. The individuals of Watareke and Matugama were clustered first (level 0.72) and then joined with Yagirala at a level of 0.66.

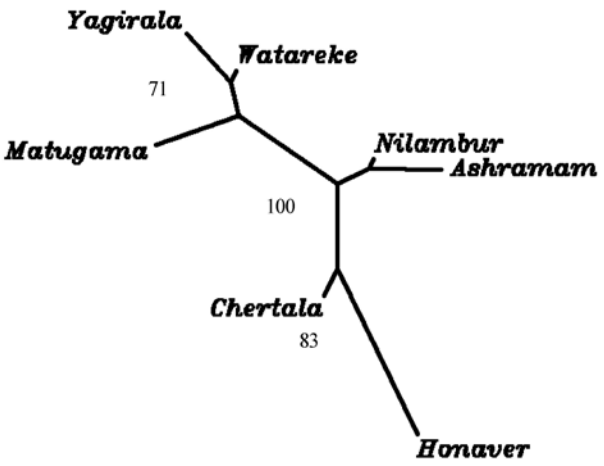

Figure 2. Unrooted phenetic tree for all populations based on genetic distance values produced from FITCH method of PHYLIP (Felsenstein, 1993). Numbers at nodes indicate the bootstrap values.

Principal co-ordinate analysis (PCOA). The PCOA chart (data not shown) clearly separated Sri Lankan populations from Indian populations. The first coordinate axis comprised the Nilambur and Chertala populations, and the populations of Honaver and Ashramam were concentrated in the second axis. The first coordinate accounted for the 13% of total variance while the second and third accounted for 11.2% and 6.3%, respectively.

Table 3. Variation explained by the first three principal coordinates.

	Axis 1	Axis 2	Axis 3
Eigen values	9.14708	4.92040	2.9357
Percent of variance	38.5846	22.2513	9.1743
Cumulative percentage	38.5846	60.8359	69.010

DISCUSSION

The multivariate analyses of morphological and molecular data indicate that all the samples belong to a single species. In the RAPD and morphometric analysis,

Table 4. Hierarchical analysis of molecular variance (AMOVA).

Variance component	d.f	SSD	MSD	Variation variance	%	P
One region						
Among population	6	405.05	67.50	6.49	34.26%	<0.001
Within population	53	660.60	12.46	12.46	65.74%	<0.001
Two region						
Region	1	153.75	153.75	3.90	18.77%	<0.001
Among population	5	251.30	50.26	4.44	21.36%	<0.001
Within population	53	660.60	12.46	12.46	59.87%	<0.999

if the populations sampled had been two distinct species, the unrooted phenetic tree and UPGMA cluster analysis would have separated them into two distinct groups. In the RAPD dendrogram, populations of Nilambur and Honaver representing *C. metzianus* mixed with populations of *C. rivalis*. Moreover, the RAPD analysis was unable to identify any species-specific markers. Morphologically, the distinction between *C. metzianus* and *C. rivalis* is that the latter has larger fruits and a fruit scale channel in the middle. The other morphological features are shared by both species. The Mantel test results indicated overall independence between the fruit morphological data and the genetic distances measured using RAPD analysis. The lack of correlation between the genetic distance and the presence/absence of fruit scales was further confirmed by the UPGMA dendrogram and PCO analysis, suggesting further that both *C. rivalis* and *C. metzianus* cannot be distinguished on the basis of fruit scales. Hence, the morphometric studies revealed a high degree of morphological similarity among populations and that no distinct phenetic gap existed between the two species; rather, the morphological characters were found to overlap. The OTUs from Nilambur and Honaver corresponding to two different populations of *C. metzianus* were found to be intermixed with the remaining OTUs representing different populations of *C. rivalis*. The lack of distinct phenetic structure in ordination analyses also indicates a high similarity between these two species. Therefore, the results of both morphometric and RAPD analyses did not provide any support to separate *C.rivalis* and *C. metzianus* into distinct species. Hence, *C. rivalis* should be merged to *C. metzianus*.

Genetic diversity

Genetic richness can be assessed by estimating the genetic diversity parameters (viz. percentage of polymorphic loci and gene diversity index) (Yeh, 2000). The percentage of polymorphic loci was 95%. The high levels of genetic polymorphism have been documented in other rattans (Bon et al., 1996) and palms such as *Euterpe edulis* (Cardoso et al., 2000). In this study, the amount of gene diversity obtained was found to be 0.18, which is similar to figures reported for outcrossing, herbaceous, and insect pollinated species (Cardoso et al., 2000). Among the seven analysed populations, the genetic diversity measures were highest in the Chertala population (0.25), followed by Nilambur (0.23) and Ashramam (0.21). The higher gene diversity of the Chertala population may be due to its high population density. Hence, an evolutionary force like genetic drift can operate on a large number of genotypes, resulting in the maintenance of a great of amount of gene diversity. This population can be considered a hot spot of genetic variation and an important reservoir of potentially useful genes, and it hence merits a high priority by conservation managements.

The results of AMOVA on RAPD data showed that the variance component among populations was 34.26%, and the variance component within populations was 65.74% of the total variation when all the populations were treated as one region. A similar pattern was also observed in the studies of *Grevillea barklyana* (Hogbin et al., 1998), and *E. grandis* (Grattapaglia et al., 1997). Studies on pines (Hamrick and Godt, 1989) and *Santalum album* (Suma and Balasundaran, 2003), on the other hand, indicated a reverse trend, in which most of the genetic diversity was found to reside among populations rather than within them. Autogamous species are generally supposed to allocate most of their genetic variability among populations, which results in little intra-populational genetic variability. This is the case with *Oryza* (Buso et al., 1998), and *Gentianella* (Fischer and Matthies, 1998), though *Hordeum spontaneum* is an exception (Baum et al., 1997). Long-lived, woody, late-successional organisms typically harbor the highest levels of genetic variation within populations (Hamrick and Loveless, 1989; Hamrich and Godt, 1989). In this study, as populations were divided into two regions, with all the Western Ghats populations in one region and the rest grouped in another, the results of AMOVA indicated that the percentages of variation attributable to the differences between regions, among populations within regions, and among individuals within populations were 18.77%, 21.36% and 59.87%, respectively. The breeding system is one of the main factors determining the genetic structuring of plant populations (Hamrick and Godt, 1989), and the considerable amount of intra-populational variation in these *Calamus* populations reflects its allogamous mode of reproduction.

The seven populations in this study covered a wide geographic range in latitudes and geographical distances. The most genetically similar populations (Yagirala and Watareke) were geographically separated by a distance of only 25 km while the genetically distant populations (Honaver and Yagirala) were separated by about 980 km. The test of correlation between the genetic and geographic distance matrices using the mantel test revealed a significant positive correlation with $r = 0.7327$ ($p = 0.0090$), which was partly supported by the UPGMA dendrogram and PCO analysis. Both analyses revealed the same tendency for the individuals to group according to geographic localities.

Diminishing forest areas coupled with overexploitation has seriously affected the wild populations of rattan, and its conservation is a major concern. Detailed knowledge about the genetic diversity within and among populations is essential to developing gene banks for *ex-situ* conservation. The populations such as Honaver, Matugama, and Yagirala analyzed in the present study are under continuous threats of degradation, mainly due to habitat alteration and irregular harvesting of stems for the furniture and handicraft industries. As a preliminary step in the *ex-situ* conservation of rattans, germplasm collections and seed stands representing all the populations have to be maintained.

CONCLUSIONS

The present study based on random amplified polymorphic DNA analysis and morphometric studies shows that *C. metzianus* and *C. rivalis* are indistinguishable. Since *C. metzianus* was reported first, this name will stand. Apparently, RAPD fingerprints can contribute significantly to our understanding of the diversity within the different populations of *C. metzianus* and *C. rivalis.*

Taxonomic treatments

Calamus metzianus Schlecht in Linnaea 26: 727. 1853; Becc. in Hook. f., Fl. Brit. India 6: 462. 1892; Becc. in Ann. Roy. Bot. Gard. Calcutta 11: 82, 221. t. 67. 1908. Renuka, Rattans of Western Ghats 53, t. 16. 1992 [Figure 6.10; Plate 4]—TYPE: India, Karnataka, Mangalore, 1847, *R.T. Hokanaker*, *1906* (holotype, FI).

Calamus rudentum Mart., Hist. Nat. Palm. 3: 340. 1823-1853.

Calamus rivalis Thw. *ex* Trim. in J. Bot. 23: 268 (1885), syn. nov.

Clustering, slender cane, climbing to a height of 10-15 m. Stem diameter with sheath 1-2 cm, without sheath to 1 cm. Leaf sheath green, armed with solitary, yellowish, horizontal or slightly deflexed spines to 10 mm long; knee present; ochrea short, deciduous. Leaves 1.5 m long; petiole short or absent; rachis acutely trigonous, bifaced and naked above; leaflets 40 × 2 cm, regular, linear, acuminate to a slender apex, lanceolate, on the upper surface mid vein with out spines or spinulose at the apex, ciliate beneath from centre upward, margin spinulose, upper leaflets small, bristly, terminal pair free at base. Inflorescences flagellate; male inflorescence 3 m. long; partial inflorescence 20-30 cm long, rachillae 3 cm long; female inflorescence 3 m long, partial inflorescence 70 cm long, rachilla 5-10 on each side, terminating in a filiform caudate Appendix, 8 cm long, primary spathe very long; narrow, closely sheathing, armed with numerous reflexed scattered spines, obliquely truncate at the mouth, prolonged in to a short triangular point; secondary spathe very narrow tubular infundibuliform; 2.5 cm length, spiny and narrow at base; male flowers 4 mm long, acute, calyx veined; female flowers 3 mm long, ovate; involucre cupular, unequal margin; fruiting perianth not pedicelliform. Fruit very broadly ovoid, 7-8 mm broad and 11 mm long; scales on 21 vertical rows, very faintly channeled, straw yellow, violet when ripe, margin erosely toothed; seed ovate, 9 mm broad and 5 mm thick Endosperm not ruminate; embryo basal.

Distribution. India, Sri Lanka. Restricted to plains along the backwaters and coasts and in sacred groves.

Phenology. Flowering: September-October; Fruiting: March-April.

Specimens examined. **INDIA.** KERALA: Malappuram Dist., Pattakkarimbu, Nilambur, 1988, *Babu 4081*, *4082*, *4083* (KFRI); Nilambur, *Perrie 49362*, *49363*, *49364*, *49365*, *49366* (CAL); Nilambur, 1984, *Renuka 3061*, *4031*, *4032*, *4033* (KFRI); Nilambur, 2002, *Sreekumar 8451*, *8452* (KFRI). Kollam Dist., Ashramam compound, 1999, *Renuka* & *Sasidharan 3443* (KFRI); Ashramam, 2001 *Sreekumar 22446* (KFRI). KARNATAKA: Uttara Kannada Dist., Honaver, *Renuka 5884* (KFRI); Honaver, *Renuka 5884* (KFRI); Honaver, *Renuka 7555* (KFRI); Honaver, 2000, *Anto* & *Sreekumar 22401* (KFRI); Honaver, 2002, *Sreekumar 8444* (KFRI); SRI LANKA: Watareke, *Neela de Zoysa 6814* (KFRI).

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Appendix. List of qualitative and quantitative characters used in the study. (Characters abbreviations included in the bracket)

No.	Character	Measurements/Status
1.	Stem diameter (stemdia)	cm
2.	Internodal length (intle)	cm
3.	Leafsheath spine length (lesle)	cm
4.	Leafsheath spine (lesp)	Sparse (0); Dense (1)
5.	Leafsheath spine hairs (lesph)	Absent (0); Present (1)
6.	Petiole (peole)	Absent (0); Present (1)
7.	Leaf length (lflen)	m
8.	Leaflet length (lfletl)	cm
9.	Leaflet width (lfwid)	cm
10.	Leaflet upper midvein cilia length (lfumcl)	cm
11.	Leaflet lower midvein cilia length (lflmcl)	cm
12.	Terminal leaflet length (terleng)	cm
13.	Terminal leaflet width (terlwid)	cm
14.	Partial Inflorescence length (parlen)	cm
15.	Number of male rachilla/partial inflorescence (nomlrach)	Number
16.	Number of female rachilla/partial inflorescence (noflrach)	Number
17.	Number of fruits per rachillae (nofrurach)	Number
18.	Male rachillae length (mlrachl)	cm
19.	Female rachillae length (flrachl)	cm
20.	Inflorescence caudex length (infcaudel)	cm
21.	Rachillae prophyll length (rachprol)	cm
22.	Rachillae prophyll width (rachprowid)	cm
23.	Fruit apical beak length (fruapl)	cm
24.	Fruit diameter (frudia)	cm
25.	Fruit length (frulen)	cm
26.	Fruit scales length (fruscal)	cm
27.	Fruit scales width (fruscalwi)	cm
28.	Fruit scales margin (frusclmar)	Reddish-Brown (0); White (1)
29.	Number of vertical rows of scales in fruit (novrowscl)	Number
30.	Fruit scales channel (fruscales)	Absent (0); Present (1)
31.	Seed length (sedlen)	cm
32.	Seed width (sedwid)	cm

由形態學及分子分析對 *Calamus rivalis* Thw. ex Trim. 及 *C. metzianus* Schlecht (Arecaceae) 分類學之重新評估

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對分佈於印度及斯里蘭卡之兩種具破壞力之植物種的族群以隨機放大之多型性 DNA 標誌 (RAPD) 及形態特性加以分析。RAPD 使用 10 個 decamer 之引子 (primer) 得到共 117 個標示 (markers) 其中 95% 具多型性。兩個族群之間之多型性基因位 (loci) 其百分比範圍為 26.5 到 68.38；而基因距離為 0.05 到 0.28。族群內之變異大於族群間之變異。上述結果顯示單一物種之存在；因此 *Calamus rivalis* 可以併入 C. metzianus。

關鍵詞：*Calamus*；樹狀圖；遺傳岐異度；隨機放大之多型性標誌。