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Genetic Diversity of *Sindora siamensis* Teijsn. Ex Miq. From Vietnam Detected by Inter Simple Sequence Repeat (ISSR) Markers

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Abstract

Sindora siamensis Teijsn. ex Miq. is a large evergreen tree and of Critically Endangered (CR) species in southern Vietnam. 60 individuals of 6 naturally distributed populations classified by habitat for this species were analyzed. Inter simple sequence repeat (ISSR) markers were employed to investigate the genetic variability in. Results showed higher at the species level (*PPB*=94.96%, H_T=0.280; I_T=0.417), but lowers at within populations, which Pop4 lowest genetic diversities (*PPB*=44.44%; H_s=0.173; I_s=0.2422) and the highest value of Pop3 (*PPB*=73.92%; H_{eS}=0.2247; I_s=0.3546). The hierarchical analysis of molecular variant revealed differentiation among population (14%), which was confirmed by the gene differentiation coefficient (G_{ST}=0.1871) and occurred gene flow. The G_{ST} value translated into corresponding low level of gene flow (N_m=2.1720). And it's showed that the migration among the six populations was low, which is 2.03%.

Keywords: *Sindora siamensis*; ISSR; Genetic analyzes; Conservation strategy; Vietnam

Introduction

Sindora siamensis Teijsn. ex Miq. is a large evergreen tree found in open semi- deciduous/green or open forest in southern Vietnam, has important ecological and commercial values. In recent decades, *S. siamensis* has been threatened by rapid habitat destruction and overexploitation of the forest for timber. It has been classified as a category "Critically Endangered (CR) "species" [1,2]. Some conservation initiatives have been initiated by the national and regional governments, and these include establishing nature reserves and conducting population surveys [1]. During the year 2012 and 2013, field identification research has identified approximately 20-50 individual plant remaining in the four regions (Yokdon Distric (Daklak Province), Phu Thien District (Gia Lai Province), Binh Chau District (Ba Ria Vung Tau Province), Tan Phu District (Dong Nai Province)), and 300-500 individual plant remaining in the two regions (Cam Ranh District (Khanh Hoa Province), Tuy Phong District (Binh Thuan Province)). Otherwise, field studies suggest that it has little seedling recruitment in its natural habitat. Moreover, habitat degradation and destruction continue in unmanaged. A better understanding of genetic variation within and among populations of rare and endangered species is essential when developing management strategies for both *in situ* and *ex situ* conservation activities [3]. However, studies on the genetic diversity of this species have not been conducted in Vietnam. Genetic variation is currently under stood as a critical variable to the long-term survival of a population or species [4,5]. Understanding the genetic variation and diversity within and among populations of rare and endangered species is essential when developing management strategies for both *in situ* and *ex situ* conservation activities [3]. Therefore, estimating inter- and intra-population genetic diversity is critical to the protection and long-term availability of *S. siamensis* both in terms of ecological biodiversity. Current research methods support the use of molecular markers as suitable and accurate tools for population genetic diversity detection.

The advantages of inter simple sequence repeat (ISSR) lies within in its low-cost use, convenience of use, and high-level of reliability in reproducing results [6-8]. ISSR methods have established widespread and accepted use for applications in population genetic studies of both wild and cultivated plants [8]. In the present study, the ISSR marker system was used to evaluate the level and structure of genetic diversity in wild *S. siamensis* populations found and distributed in their natural habitats for conservation and sustainable utilization. The objects of this study were as follows: (1) to estimate genetic diversity; (2) to analyze genetic relationships and differentiation among populations and (3) to contribute and catalogue the data of this study for use in the conservation and sustainable utilization of the researched plants within Vietnam.

Materials and Methods

Plant materials

For genetic structure studies, 6 geographically different populations of 60 individuals of *S. siamensis* from southern Vietnam [Yokdon District (Daklak Province), Phu Thien District (Gia Lai Province), Cam Ranh District (Khanh Hoa Province), Tuy Phong District (Binh Thuan Province), Binh Chau District (Ba Ria Vung Tau Province), Tan Phu District (Dong Nai Province)] were sampled. Each population was positioned by GPS with location details listed in Table 1 and Figure 1. Young leaf tissues were collected from each sampled individual plant located at least 300 m apart and dried in silica gel for DNA extraction. Each sample was preserved at a constant -20°C for DNA analysis.

DNA extraction and ISSR-PCR amplification

CTAB method with modification of adding 10%SDS was used to DNA extraction. A screen of the 60 accessions using 12 selected ISSR primers was used for the analysis. PCR amplification was repeated to check the stability and reproducibility of ISSR DNA fingerprinting.

Each 20 µl reactions consisted of 2 mM $MgCl_2$, 0.25 mM each of dNTPs, 1U Taq DNA polymerase, 0.2 µM primer and approximately 30 ng DNA templates from each individual. The amplifications were

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Population	Geographic localities	Longitude/ Latitude	Altitude (m)	Forest types
Pop1	Yokdon Distric (Daklak Province)	12º93'029" N, 107º71'234" E	186	Deciduous forest
Pop2	Phu Thien District (Gia Lai Province)	13º58'047" N, 108º23'864" E	286	Deciduous forest
Pop3	Cam Ranh District (Khanh Hoa Province)	12º07'459" N, 109º19'163" E	34	Open forest near the sea
Pop4	Tuy Phong District (Binh Thuan Province)	11º22'073" N, 108º64'931" E	39	Open forest near the sea
Pop5	Binh Chau District (Ba Ria Vung Tau Province)	10º54'754"N, 107º48'446" E	31	Semi-deciduous forest near the sea
Pop6	Tan Phu District (Dong Nai Province)	11º09'936"N, 107º40'503" E	163	Evergreen forest

Table 1: Details of *S. siamensis* genotypes and populations from Vietnam used in study.

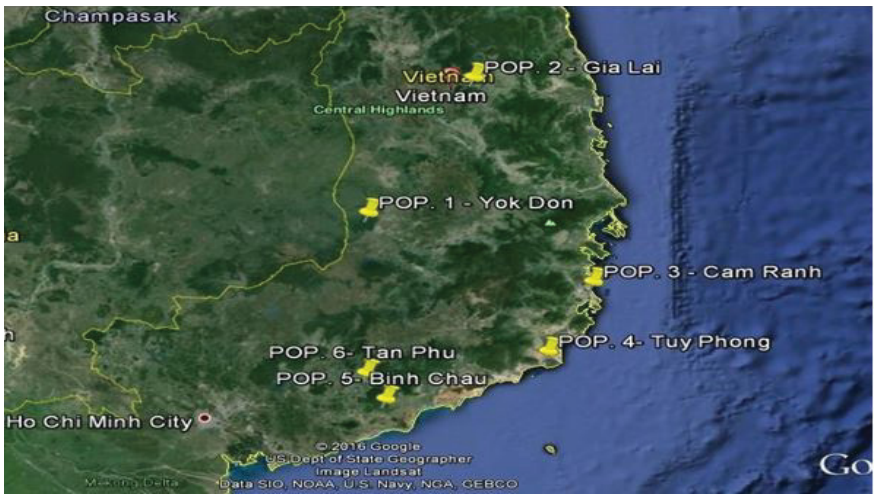

Figure 1: Geographic localities of *S. siamensis* populations in South of Vietnam.

performed in a Peqstar 96X Universal Gradient thermocycler with the following conditions: initial denaturation at 94 0C for 5 min; 10 cycles of 94°C for 45 s, annealing temperature +5 (Ta +5)°C for 45 s, decreased 0.5°C/cycle, 720C for 1min 30 s; 36 cycles of 94°C for 45 s, annealing temperature for 45 s, 72°C for 1 min 30 s; Final extension at 72°C for 15 min; the amplification products were separated in 2% agarose gel, using TBE buffer at 60 V for 3 hours, stained with ethidium bromide (0.5 µg/ml), and photographed under 254/312 nm wavelength lights using Micro Doc Gel Documentation System (Cleaver Scientific, USA).

Data analysis

ISSR was performed by following the methodology [9]. Microsoft Office Excel 2007 was used to estimate genetic diversity parameters [10].

The computer program POPGENE [3] was used to calculate the statistics of genetic variation for the isizyme data based on individual genotypes for each population. The estimates including the percentage of polymorphic loci (*PPB*), expected genetic heterozygosity (H_{eS}), the Shannon index (*I*); the level of gene flow (*Nm*) [11,12]. Genetic distance between populations (*D*). Inter- population's genetic diversity (H_S), total genetic diversity (H_{eT}) and Nei's coefficients of genetic differentiation (GST) were calculated using the Popgene 32 software [13].

The AMOVA was used to describe variance components and their significance levels for variation among individuals within and among the populations. Similarity coefficient between pair of samples and UPGMA dendrogram for genetic relationship was calculated and established by using NTSYSpc 2.1 software [14,15].

Results

Genetic diversity

The present study revealed the genetic diversity within a collection of *S. siamensis* germplasm representing different geographical regions of Vietnam, using molecular (ISSR). Out of total twelve that produced clear and reproducible bands were selected for further analysis. These are 12 selected primers generated totally primers 139 scorable bands were varied between 10 (17899B) and 14 (UBC 856T), with an average of 11.6. The data were utilized for further computations. Out of 139 bands, 36 bands shared by all investigated populations, 22 bands were monomorphic for only one population (14 of them were monomorphic with population 4), 18 bands were absent in only one population (12 of them were absent with population 4) (Table 2).

Very few polymorphism and low genetic diversity were detected for six populations at the population level. Genetic diversities for each *S. siamensis* population were estimated in Table 3. Among six populations examined in this study, Pop4 maintained lowest genetic diversities (*PPB*=44.44%; H_{eS}=0.173; I_S=0.2422); the highest value of PPB%, H_{eS} and I_s was found in Pop3 (*PPB*=73.92%; H_{eS}=0.2247; I_S=0.3546). The mean genetic variations of six *S. siamensis* populations were high value (*PPB*=94.96%, H_{eT}=0.280; I_T=0.4171).

Genetic structure

The genetic differentiation (G_{ST}) among populations was estimated 0.1871, which is indicated that 18.71% of the genetic variability was distributed among populations, and 81.29% of the variation existed within population. The number of migrants (*Nm*) was estimated at 2.1720 individuals per generation between populations, which is indicating that there a high migration rate between populations. The genetic distance (D) for every pairwise comparison between each population was estimated in Table 4. The highest genetic distance was 0.1689 between population Pop3 and Pop4, while the lowest (0.0325) occurred between Pop2 and Pop5.

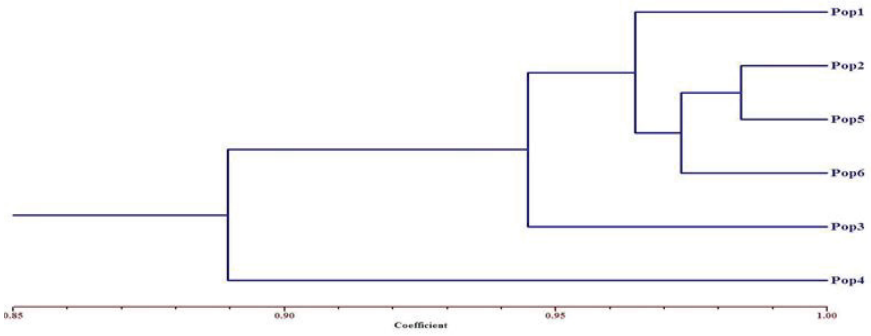

Figure 2: Dendrogram for genetic relationship among studied *S. siamensis* populations.

Primer code	Sequence (5' → 3')	T_a (°C)	Size range	PPB (%)					
				Pop1	Pop2	Pop3	Pop4	Pop5	Pop6
UBC807	5'-$(AG)_8$ T-3'	54	200-1200	61.54	76.92	92.31	15.38	100.00	46.15
UBC826	5'-$(AC)_8$ C-3'	54	300-1000	81.82	54.55	54.55	81.82	45.45	54.55
UBC856C	5'-$(AC)_8$ CA-3'	52	300-1200	72.73	72.73	90.91	45.45	90.91	81.82
UBC856T	5'-$(AC)_8$ TA-3'	52	200-1900	78.57	57.14	57.14	50.00	78.57	71.43
UBC873	5'-$(GACA)_4$ -3'	52	300-2000	83.33	58.33	66.67	33.33	50.00	50.00
844A	5'-(CT)8 AC-3'	52	200-1200	61.15	46.15	84.62	38.46	53.84	76.92
HB10	5'-$(GA)_6$ CC-3'	52	250-900	70.00	70.00	80.00	30.00	70.00	30.00
HB12	5'-$(CAC)_3$ GC-3'	52	250-1200	91.67	83.33	83.33	33.33	75.00	100.00
808	5' -$(AG)_8$ C-3'	52	300-1500	27.27	54.55	36.36	27.27	63.64	45.45
17898A	5' -$(CA)_6$ AC-3'	54	200-1300	72.73	54.55	100.00	72.73	63.64	72.73
17898B	5' -$(CA)_6$ GT-3'	54	200-2500	72.73	72.73	63.64	45.45	81.82	63.64
17899B	5'-$(CA)_6$ GG-3'	54	200-1500	90.00	80.00	70.00	60.00	90.00	90.00
Average	--	--	--	71.96	65.08	73.92	44.44	71.91	71.96

Table 2: ISSR primers used in this study.

Genetic diversity	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6
Average expected heterozygosity (H_{eS})	0.243	0.241	0.247	0.173	0.258	0.234
Percentage of polymorphic loci *PPB* (%)	71.96	65.08	73.29	44.44	71.91	65.22
Shannon's information index (*I*)	0.3480	0.3413	0.3546	0.2422	0.3676	0.3354

Table 3: Genetic diversity estimate of 6 investigated populations based on ISSR markers.

Population	Pop2	Pop3	Pop4	Pop5	Pop6
Pop1	0.0554	0.0804	0.1252	0.0512	0.0496
Pop2	--	0.0506	0.1308	0.0325	0.0474
Pop3	--	--	0.1689	0.0663	0.0948
Pop4	--	--	--	0.1074	0.1206
Pop5	--	--	--	--	0.0388

Table 4: Genetic distance between populations of *S. siamensis*.

Source of variation	*d.f.*	Sum of squares	Mean of squares	Variance components	Ratio of variance (%)	*p*-value
Among Populations	5	218.383	43.677	2.683	14%	<0.001
Within Populations	54	909.500	16.843	16.843	86%	<0.001

Table 5: AMOVA analysis of 60 individuals of six populations of *S. siamensis* using ISSR makers.

The UPGMA cluster analysis clustered all six populations into 3 groups, which corresponded to their geographic origins (Figure 2). The Pop4 seems to be isolated out of other, which possesses the low genetic diversity. The genetic distances between it and other were large.

AMOVA analysis revealed remarkable genetic different among all 6 populations, with 14% of total genetic variability portioned among populations and 86% of total genetic variability portioned among individuals within populations (Table 5).

Discussion

The ISSR survey of six populations of *S. siamensis* revealed a large variation in *P*, with values ranging from 43.44% (Pop1) to 71.96% (Pop2). The results of present study using ISSR makers revealed low level of genetic diversity within populations and remarkable genetic different among populations. Population genetic diversity in species is affected by a number of evolutionary factors including mating system gene flow and seed dispersal, geographic range as well as natural [16]. Otherwise, the geographic range of a species appears to influence the levels of genetic diversity within populations greatly. In general, among the breeding Population genetic diversity in species is affected by a number of evolutionary factors including mating system gene flow and seed dispersal, geographic range as well as natural selection [16]. Otherwise, the geographic range of a species appears to influence the levels of genetic diversity within populations greatly. In general, among the breeding system groups, selfing taxa usually possess lower genetic diversity within population's taxa with seeds that disperse only by gravity had lower values [17-19]. This is implied that a large proportion of genetic variation was partitioned among populations, which was classified by habitat and self-pollinated species. Nybom reported that

the mean within population genetic diversity (H_{eS}) for self-pollinated species, mixed-mating species and outcrosses were 0.41, 0.60 and 0.65, respectively; the mean within population genetic diversity (H_{eS}) for gravity dispersed species, attaching species and win, water ingested were 0.47, 0.56 and 0.61, respectively [19]. It is conspicuously low when compared with the values of other seed plants with similar life history characteristics in Nybom and is nearly equivalent to that of self-pollinated and gravity dispersed species [19]. This study suggests that the genetic diversity within populations of *S. siamensis* is conspicuously low when compared with values of other seed plants with similar life history characteristics in Nybom [19]. Evolution Potential and ability of trees against adverse environment depend on the genetic variation within populations [20]. The AMOVA indicated that 14% of the total genetic variation partitioned among populations of *S. siamensis*, which indicated that *S. siamensis* might be a heterologous plant [16]. The low genetic differentiation among population and geographical distance suggested that *S. siamensis* had low geographic differentiation, which may be due to similar selection pressure from different locations, or in sufficient interference of geographic factors towards gene follow among population [20]. Although the results of this study may suggest an inbreeding in self- compatible *S. siamensis*, which is not supported by genetic structure populations (G_{ST}=0.247) and does not correspond to self-pollinated populations (G_{ST}=0.590) [19]. However, the G_{ST} value is more similar to those of mixed-mating species (G_{ST}=0.200) or outcrosses (G_{ST}=0.220) [19]. Nei's genetic diversity analysis demonstrated a similar pattern of genetic structure, which is similar the average obtained for mixed-mating species (G_{ST}=0.200) [19]. The N_m of *S. siamensis* among population was 2.1720, which is indicated that there was a certain extent of genetic differentiation among populations.

Also using the ISSR maker, Yang et al. investigated 11 natural *Sindora glabra* populations from Hainan Island in China and indicated that the percentage of polymorphic bands was 93.4%, the Nei's gene diversity was 0.321, Shannon's information index was 0.482, and gene differentiation coefficient was 0.1944, these revealed a high level of genetic diversity maintained in *S. glabra* populations [20]. Comparing to these parameters, the achieved genetic diversity and gene differentiation of six populations from Vietnam in current research was slight lower.

The population differentiation of *S. siamensis* may be explained by factors such as historical processes such as long-term isolation or habitat fragmentation. Because the historical factors are may influence the distribution and partitioning of the genetic diversity in plant species [21]. Beside from these historical reasons, the gene flow/seed dispersal pollinator activities, breeding system and habitat destruction are also significant factors that have determined the distinct genetic. Based on our field observations, the populations of *S. siamensis* cover a large geographical area, geographic isolation was found in deciduous forest (Yordon, Phu Thien), semi-deciduous (Binh Chau); green forest (Tan Phu), or open dwarf forest (Cam Ranh, Tuy Phong) in southern Vietnam. Thus, populations varied significantly in physical conditions, such as topography, rainfall and temperature (Table 1). There are major factor influencing genetic differentiations by limiting the amount gene follow via both pollen and seeds [22]. Different physical conditions can lead to fruit ripening and flowering asynchrony, the latter of which in turn results in the substantial decrease of lack of gene follow via pollen dispersal [23-26]. *S. siamensis* has a restricted gene flow due to limited pollen and seed dispersal.

Conclusion

The understanding on population genetic variability is essential to effective conservation and sustainable management. The overall genetic diversity in *S. siamensis* populations was found to be low proportion of the total genetic diversity when analyzed separately (44.44-73.92%) or together (94.96%). Thus, although plants from three of the six populations (Pop1, Pop5 and Pop6) have been brought into nature reserves, not all of the *in situ* or *ex situ* populations contain the representative genetic diversity of the species. Therefore, the extant *in situ* populations should be fully conserved to prevent further loss genetic diversity. It would be suggestion to preserve the most genetically divergent population that possesses more specific, locally adapted genotypes as preferential source populations in the *ex situ* conservation program.

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References

1. Nghia NH (1999) Biodiversity conservation. Agriculture Published House, Vietnam. p: 148.
2. Vietnam Academy of Science and Technology (2007) Vietnam Red Data Book, Part II. Plants.
3. Hogbin PM, Peakall R (1999) Evaluation of the contribution of the genetic research to the management of the endangered plant Zieria prostrates. Conserv Biol 13: 14-522.
4. Anatonovis J (1984) Genetic variation within population. In: Dirzor R, Sarukan J (eds) perspectives on plant population biology. Sinauer, Sunderland. pp: 229-241.
5. Beardmore JA (1983) Extinction, survival and genetic variation. In: Schoenwald-Cox CM, Chamber SM, Macbryde B, Thomas L (eds). Genetics and Conservation. Benjamin-Cummings, Menlo Park, pp: 125-151.
6. Lu X, Liu L, Gong Y, Zhao L, Song X, et al. (2009) Cultivar identification and genetic diversity analysis of broccoli and its related species with RAPD and ISSR markers. Science Horticulture 122: 645-648.
7. Nagoaka T, Ogihara Y (1997) Applicability of inter-simple sequence repeat polymorphism in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor Appl Genet 93: 133-139.
8. Roy SC, Chakraborty BN (2009) Genetic diversity and relationships among tea (Camellia sinensis) cultivars as revealed by RAPD and ISSR based fingerprinting. Indian Journal Biotechnol 8: 370-376.
9. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18: 31-65.
10. Vicente MCD, Lopez C, Fulton T (2004) Genetic diversity analysis with molecular marker data: Learning module. International Plant Genetic Resources Institute (IPGRI) and Cornell University, USA.
11. Slatkin M (1985) Gene flow in natural populations. Annu Rev Ecol 16: 393-430.
12. Mcdermott JM, Mcdonald BA (1993) Gene flow in plant pathosystems. Annu Rev Phytopathol 31: 353-373.
13. Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Nat Acad Sci USA 70: 3321-3323.
14. Rohlf FJ (2004) NTSYSpc-Numerical taxonomy and multivariate analysis system Version 2.1-User Guide. Applied Biostatistics Inc, USA.
15. Peakal R, Smouse PE (2006) GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6: 288-195.
16. Hamrick JL, Godt MJW (1989) Allozyme iversity in plant species: In: Brown AHD., Clegg MT, Kahler AL, Weir BS (eds). Plant population genetics, breeding and germplasm resources. Sinauer Associates, Sunderland. pp: 43-63.
17. Zhang LJ, Dai SL (2010) Genetic variation within and among populations of Orychophragmus violaceus (Cruciferae) in China as detected by ISSR analysis. Genet Res Crop Evol 57: 55-64.
18. Huang HW, Ai B, Kang M (2014) Assessment of genetic diversity in seed plants based on uniform criterion. Molecules 19: 20113-20127.

19. Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol Ecol 13: 1143-1155.

20. Yang JC, Li QQ, Yu N, Yin GT, Wu ZF, et al. (2016) Genetic diversity and structure among natural populations of Sindora glabra in Hainan Island, China as revealed by ISSR markers. Bioch Syst and Ecol 69: 145-151.

21. Parks CR, Wendel JF, Swell MM, Qiu YL (1994) The significance of allozyme variation and introgression in the Liriodendron tulipifera complex (Magnoliaceae). Am J Bot 81: 878-889.

22. Pfeifer M., Jetschke G (2006) Influence of geographical isolation on genetic diversity of Himantoglossum hircium (Orchidaceae). Folia Geobot 41: 3-20.

23. Li MM, Cai YL, Qian ZQ, Zhao GF (2009) Genetic diversity and differentiation in Chinese sour cherry Prunus pseudocerasus Lindl., and its implications for conservation. Genet Resour Crop Evol 56: 455-464.

24. Ferrante M, Yeh JC (2010) Head and flux variability in heterogeneous unsaturated soils under transient flow conditions. Water Resour Res 5: 1471-1479.

25. Robichaux RH, Friar EA, Mount DW (1997) Molecular genetics consequences of a population bottleneck associated with reintroduction of the Mauna Kea Silverwoold (*Argyro xiphium* sanwicense spp. sanwicense L. [Asteraceae]. Conserv Biol 11: 1140-1146

26. Weising K, Nybom H, Wolff K, Kahl G (2005) Fingerprinting in plants: Principles, methods, and applications (2nd edn). CRC Press Taylor & Fancies Group, USA.