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Kuruna debilis, microsatellites, population genetics, Sri Lanka

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RESEARCH ARTICLE

Genetic diversity and population structure of the threatened temperate woody bamboo *Kuruna debilis* (Poaceae: Bambusoideae: Arundinarieae) from Sri Lanka based on microsatellite analysis†

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Abstract: Species of the temperate woody bamboo genus *Kuruna* Attigala, Kathriar. & L.G. Clark (Poaceae: Bambusoideae) distributed in Sri Lanka and southern India, are threatened due to deforestation and habitat fragmentation. The current study focused on the tetraploid woody bamboo *Kuruna debilis* (Thwaites) Attigala, Kathriar. & L.G. Clark, using twelve variable microsatellite loci to assess the genetic diversity and population structure in six known Sri Lankan populations. Due to the rarity of the species, an exhaustive sampling of accessible plants resulted in a total of only 28 individuals. Nonetheless, the allelic diversity was high at most loci and given the limited distances separating populations (< 65 km apart), they exhibited a fairly high genetic differentiation (F_{ST} = 0.113) and strong isolation by distance. Structure, neighbour-joining, and neighbour-net analyses concur in grouping the six *K. debilis* populations into three genetic clusters consistent with the spatial proximity of the populations: one cluster comprised populations from the Piduruthalagala Mountain and Horton Plains, the second cluster consisted of the population from Adams Peak and the last comprised the populations from the Handapan Ella Plains. Due to multiple indicators of high allelic diversity, the population from the northern Horton Plains (LA124) should be targeted for conservation. Moreover, the population found in Adams Peak (LA159) is also genetically important and critical to the conservation of these species due to its unique genetic diversity. As the first population genetics study of Bambusoideae in Sri Lanka, we anticipate that our results will provide a foundation for future comparative population genetics and conservation studies in the country.

Keywords: *Kuruna debilis*, microsatellites, population genetics, Sri Lanka.

INTRODUCTION

Bamboos (subfamily Bambusoideae, Poaceae) are an essential component of forest and tropical high altitude grassland ecosystems worldwide (Soderstrom & Calderón, 1979; Judziewicz *et al*., 1999; Clark *et al*., 2015). In Sri Lanka, bamboos occur naturally in all three major climatic zones (Wet, Dry and Intermediate); however, no native bamboo is found in extremely dry areas (Kariyawasam, 1998). Bamboo, in general, is an economically, culturally and ecologically important plant for Sri Lanka (De Zoysa & Vivekanandan, 1994; Gunatilleke *et al*., 1994). Most of the non-native bamboos are used in housing and construction due to their enduring, versatile and highly renewable nature. There are no statistics on bamboo consumption, but the forestry sector master plan (FSMP) of Sri Lanka estimated that the total annual consumption was at least 80,000 m^3, i.e. about 700,000 culms two decades ago (FSMP, 1995). Although the native bamboos are not of economic importance, their ecological value is significant. One such example is the animal biodiversity associated with the native bamboos such as the Sambar deer, many insects and fungi (Abayasinghe *et al*., 2014; *personal observations*). Bamboo studies conducted in Sri Lanka have mainly focused on its reproductive ecology (Ramanayake & Yakandawala, 1995; 1998; Ramanayake & Weerawardene, 2003), vegetative propagation (Ramanayake *et al*., 2001; 2007) and growth and development (Rajapakse, 1992; Ramanayake *et al*., 2001).

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Bamboos, with over 1500 species worldwide, are classified in three tribes, the tropical woody Bambuseae, the temperate woody Arundinarieae and the herbaceous Olyreae (Clark *et al*., 2015). A recent study revealed that all of the native Sri Lankan temperate woody bamboos along with the south Indian species form a major lineage in the Arundinarieae resulting in the recognition of the genus *Kuruna* (Attigala *et al*., 2014). To date, there are seven species in this genus including two endemic Sri Lankan species [*K. scandens* (Soderstr. & R.P. Ellis) Attigala, Kathriar. & L.G. Clark and *K. serrulata* Attigala Kathriar. & L.G. Clark], one species endemic to south India [*Kuruna wightiana* (Nees) Attigala, Kathriar. & L.G. Clark], and four species [*K. debilis*; *K. densifolia* (Munro) Attigala, Kathriar. & L.G. Clark; *K. floribunda* (Thwaites) Attigala, Kathriar. & L.G. Clark; *K. walkeriana* (Munro) Attigala, Kathriar. & L.G. Clark] occurring in both Sri Lanka and South India (Seethalaksmi & Muktesh Kumar, 1998; Muktesh Kumar, 2011; Attigala *et al.*, 2014; 2016).

The indigenous flowering plants of Sri Lanka include about 3,156 species (Wijesundara *et al*., 2012). Nearly one fourth of these are endemic and concentrated in the humid southwestern quarter of the country (Gunatilleke & Gunatilleke, 1990). For many years, forests in Sri Lanka have been cleared both legally and illegally, due to the rapidly increasing demand for land for settlement schemes, timber production, economic and agricultural developments and weak enforcement of land use policies in the country (Gunatilake, 1998; Government of Sri Lanka, 2000; Bandaratillake & Fernando, 2003). Several studies have reported that the closed-canopy forest cover has decreased from 84 % in 1884 to approximately 19 % in 2005 (Nanayakkara, 1996; FAO, 2005). Thus, deforestation can negatively influence the survival of the threatened and endemic flora and fauna in Sri Lanka. Many plant molecular studies have shown that habitat fragmentation and small population size may negatively influence the genetic diversity of populations (Ellstrand & Elam, 1993; Fischer & Matthies, 1998; Luijten *et al*., 2000; Paschke *et al*., 2002). Low levels of genetic diversity typically limit the ability of a population to adapt to adverse environmental conditions or increased competition (Fischer *et al*., 2000; Pluess & Stocklin, 2004). Each of the *Kuruna* species found in Sri Lanka has a limited distributional range (populations < 65 km apart) and therefore, may be under severe threat due to deforestation and habitat fragmentation. Of the six native *Kuruna* species, only *K. debilis* has several, spatially distinct populations in the understory of the upper cool mountain slopes of Sri Lanka's Central Province (elevation: 1500 – 2500 m). Based on herbarium records *K. debilis* populations are found at the Adams Peak, Horton Plains, Piduruthalagala Mountain, Knuckles Mountains and the Handapan Ella Plains of Sri Lanka. Muktesh Kumar (2011) reported that *K. debilis* has also been located recently from the Kerala part of the Western Ghats, but provided no documentation. The other five species (*K. densifolia*, *K. floribunda*, *K. scandens*, *K. serrulata* and *K. walkeriana*) are each restricted to 1 – 2 populations on a few mountain summits and in open montane grasslands in Sri Lanka (Figure 1). Due to their restricted distribution and habitat loss related to human activities, these five species are already at risk. Population genetic studies are essential for planning conservation strategies for these *Kuruna* species. Such studies provide conservation managers with significant information concerning elevated levels of random genetic drift and inbreeding, and reduced inter-population gene flow and genetic estimates of these processes could potentially be used to initiate conservation planning (Ellstrand & Elam, 1993; Young *et al*., 1996).

The primary objective of this study was to assess the genetic diversity and population structure in six natural populations of the tetraploid woody bamboo *K. debilis* in Sri Lanka.

METHODOLOGY

Population sampling

Leaf samples were collected from 28 individual plants from 6 different geographic localities, mainly from 3 remote montane forests and an open montane grassland (Figure 1). Of the 5 main localities of *K. debilis* populations (Adams Peak, Horton Plains, Piduruthalagala Mountain, Knuckles Mountains and Handapan Ella Plains), the authors were able to collect population samples from all localities except the Knuckles Mountains. Although a collection trip was made to Knuckles Mountains we were unable to locate any *K. debilis* populations. *Kuruna debilis* has a pachymorph rhizome system resulting in densely packed culms that can occupy a relatively large area. Due to clonal propagation, it is difficult in the field to differentiate individuals at a particular locality because a simple 'head count' may not reveal the true number of genets, or genetic individuals in a population. Therefore, samples were collected from individuals that were readily distinguishable as a single plant and all propagating clones of an individual plant were considered as a single entity to allow for a number of statistically significant results.

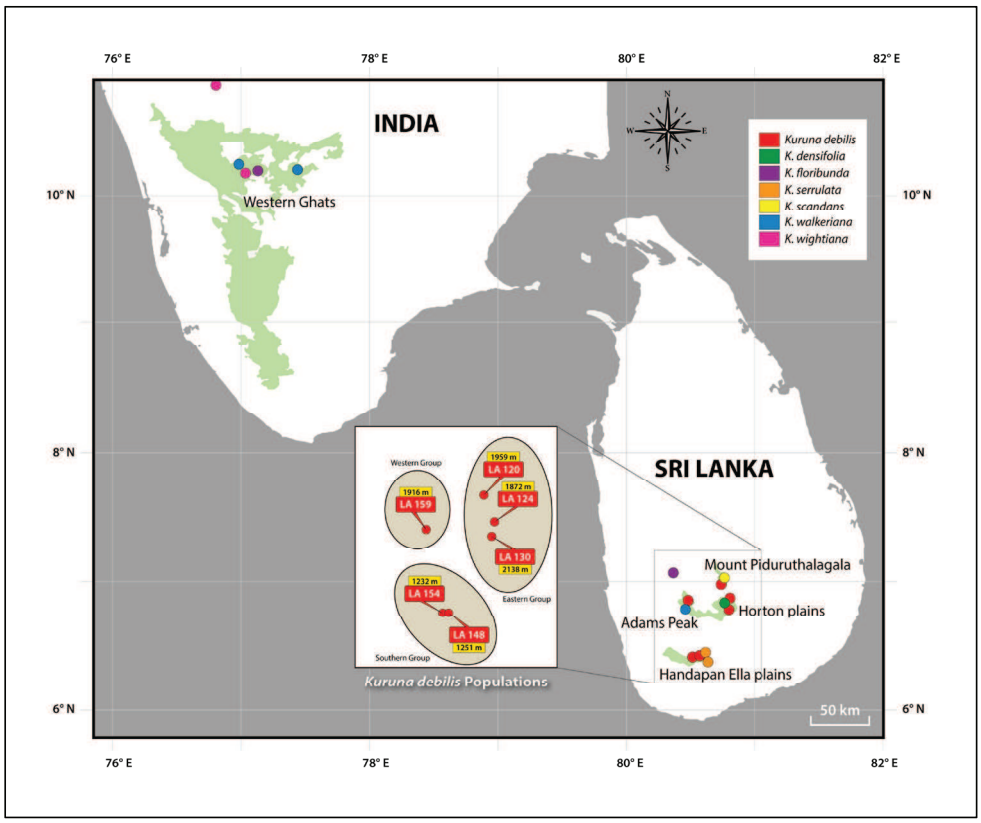

Figure 1: Distribution of Sri Lankan *Kuruna* species. Colours indicate different *Kuruna* species. The numbers in each box indicate the elevation for that population and the six *K. debilis* populations with their genetic clustering.
Map source: Wikipedia (*http://en.wikipedia.org/wiki/Sri_Lanka#mediaviewer/File:Topography_Sri_Lanka.jpg*)

Marker selection

Microsatellite sequences (SSRs) are highly polymorphic and readily replicable markers, evenly distributed throughout eukaryotic genomes. Based on previous studies on temperate woody bamboos (Kitamura *et al*., 2009; Zhan *et al*., 2009), 25 primer pairs were tested with a subset of samples to evaluate successful amplification. Of the 25 microsatellite primers, 12 were successfully amplified and selected for the study (Table 1).

DNA extraction and genotyping

Total genomic DNA extractions were performed from silica gel-dried specimens using the Iowa State University DNA Facility's Autogenprep 740 DNA extraction robot. Genotyping was performed on an ABI 3730 DNA analyser (Perkin-Elmer, Applied Biosystems Division, Norwalk, Connecticut) by the DNA Sequencing Facility at the Iowa State University. All PCR and cycle-sequencing reactions were performed in an MJ Research PTC-200 thermal cycler. PCR was performed in 25 µL volumes. The amplification products were cleaned using polyethylene glycol (PEG 6000) precipitation to remove unincorporated primers and dNTPs from the PCR products. Genotyping of microsatellite loci were subjected to standard error checking procedures, as described in DeWoody *et al*. (2006).

Individual tetraploid genotypes were scored from the electropherograms following the microsatellite DNA allele counting-peak ratios (MAC-PR) method of Esselink *et al*. (2004) using the GeneMapper® v4.1 (Applied Biosystems) software.

Table 1: Microsatellite markers used for PCR amplification

Locus	Repeat motif	Primer sequence (5'–3')	5'-Label	Allele size range (bp)	PCR parameters	Reference
Sasa500	$(AT)_n$	F: GCAGATTGCCGGTTGTTTAG R: GGAGGGCCAAGAGGTTACA	PET	297 – 355		
Sasa718	$(CT)_{in}$	F: CCCTGCAACCTTCACTCCTACA R: CCCTGCAACCTTCACTCCTACA	VIC	132 – 144	95 °C, 15 min; 35 × (94 °C, 30 s, 57 °C, 90 s, 72 °C, 60 s); 60 °C, 30 min	Kitamura *et al*., 2009
Sasa11E	$(AC)_n$	F: (TC)6(AC)5 R: ATATTGTTTGCCTGACCTACA	FAM	268 – 337		
FAN11	$(AG)_n$	F: GCAATCGCGGAGTAAAGAA R: TAAGCACACAGCAGCCAGTAGG	HEX	177 – 187	94 °C, 3 min; 35 × (94 °C, 15 s, 58 °C, 30 s, 72 °C, 45 s); 72 °C, 7 min	
FAN20	$(AG)_n$	F: GAGGGCGAGAGGTTTGAGGAATGG R: AGGACGAACGGAGGAGGAAGCACT	HEX	137 – 141	94 °C, 3 min; 35 × (94 °C, 15 s, 66 °C, 30 s, 72 °C, 45 s); 72 °C, 7 min	
FAN21	$(AG)_n$	F: CGATACTACTAGCTGGGAGGAAG R: GAGGAAAGCGAACACCAGC	FAM	164 – 190	94 °C, 3 min; 35 × (94 °C, 15 s, 62 °C, 30 s, 72 °C, 45 s); 72 °C, 7 min	
FAN27	$(AG)_n$	F:ACCCACAAGGGAGAGAGAG R:TCCTTCCCATTTCGGAGCC	HEX	14 – 152	94 °C, 3 min; 35 × (94 °C, 15 s, 58 °C, 30 s, 72 °C, 45 s); 72 °C, 7 min	
FAN26	$(AC)_n(AG)_n$	F:CGTTCCAGCGCTTCCA R:GTCCACCCACGCCTTCAC	FAM	152 – 168		Zhan *et al*., 2009
FAN16	$(AAG)_n$	F:CAGAGCTTCTGCCATTCCTTC R: GTTGTCCACCATCAGACGC	FAM	223 – 226		
FAN28	$(CA)_n(TG)_n$	F: TCCAAACCCTAATCCCCTTCAATC R: ACCCGGTCGCAACTTATCCACT	FAM	94 – 108	94 °C, 3 min; 35 × (94 °C, 15 s, 60 °C, 30 s, 72 °C, 45 s); 72 °C, 7 min	
FAN29	$(CCG)_n$	F:ACGAATCCCCAAGCCTCCTC R:TTGCCAACGTCTTCTGTGC	FAM	135 – 149		
FAN30	$(TG)_n$	F: CTTCGCGTTTGGTTTTCTGTCCTT R: TGCGGCCAAAACTACTCCCTAATC	HEX	97 – 101		

Genetic data analysis

The software SPAGeDi 1.4c (Hardy & Vekemans, 2002) was used to compute the percentage of missing genotypes and the following estimates of genetic diversity: the number of alleles per locus (N_A); the effective number of alleles (N_{Ae}) estimated following Nielsen *et al*. (2003); rarefied allelic richness (A_R) expressed as the expected

number of alleles among k gene copies (12 gene copies for the current study); and the expected heterozygosity corrected for sample size (H_e). These measures and the inbreeding coefficient (F_{IS}) were estimated for each locus and each *K. debilis* population. The A_R used in the current study is corrected for variation in sample size by using the rarefaction method recommended for uneven sample sizes (Hurlbert, 1971; Petit *et al*., 1998). Further, global F-statistics (F_{IT}, F_{IS} and F_{ST}) were estimated over all loci and populations using SPAGeDi 1.4c (Hardy & Vekemans, 2002) with the significance of average values determined by permutation (10,000 replicates). The observed heterozygosity (H_o) was calculated manually for each population over all loci.

The spatial genetic structure was inferred using a Bayesian clustering approach, which was implemented in Structure 2.3.4 (Pritchard *et al*., 2000). The model parameters were set to admixture with correlated allele frequencies between populations, and 20 replicated runs were performed for each value of K (the number of clusters) spanning from 1 – 5. The burn-in was set to 100,000 followed by 200,000 recorded Markov Chain Monte Carlo steps. Each run estimated the log probability of data, L(K). Following Evanno *et al*. (2005), the differences in log L(K) for successive values of K (ΔK) were used to determine the most likely number of clusters, a process implemented using the Structure Harvester (Earl & vonHoldt, 2012). The preferred value of K using this method is the one associated with the highest value of ΔK. The K repetitions were permuted in the software CLUMPP 1.1.2 (Jakobsson & Rosenberg, 2007) and results were graphically represented using the software DISTRUCT 1.1 (Rosenberg, 2004).

Population differentiation was assessed by analysis of molecular variance (AMOVA) using the software ARLEQUIN v3.5.1.2 (Excoffier *et al*., 2005). The K optimal genetic clusters detected with Structure determined the hierarchical levels in the AMOVA analysis and resulted in three estimates of genetic differentiation: Φ_{ST}, genetic differentiation among subpopulations relative to the total population; Φ_{SC}, genetic differentiation among subpopulations within a genetic cluster; and Φ_{CT}, genetic differentiation among genetic clusters relative to the total population. The significance of Φ-values was determined by permutation (10,000 replicates). Isolation by distance (IBD) was evaluated by assessing the correlation between the log transformed genetic distance measure F_{ST} / (1-F_{ST}) (Rousset, 1997) and geographic distance, with significance determined by the Mantel test in the programme IBDWS 3.22 (Jensen *et al*., 2005). For the IBD analyses, geographic distances were calculated manually. The significance was based on 10,000 permutation replicates. For both AMOVA and IBD analyses, the tetraploids were treated as diploids (Saltonstall, 2003) as there was no evidence of inbreeding within populations of *K. debilis* and also the analysis programmes allowed only diploid or haploid data. The diploid data matrix was generated by randomising the genotypes within each population.

A rooted majority rule consensus tree was constructed using the neighbour-joining (NJ) method with Cavalli-Sforza and Edward's chord distance (Cavalli-Sforza & Edward, 1967). The distances and the NJ tree were generated using a combination of SEQBOOT, GENDIST, NEIGHBOUR, and CONSENSE modules in PHYLIP v. 3.695 (Felsenstein, 1989). The temperate woody bamboos *Oldeania alpina* (K. Schum.) Stapleton and *Chimonocalamus montanus* J.R. Xue & T.P. Yi were used as outgroups to root the NJ tree. The resulting NJ tree was visualised with FigTree software v1.4 (*http://tree.bio.ed.ac.uk/software/figtree/*).

As further means of visualising the genetic clustering of sample populations, a network-building distance-based algorithm (Neighbour-Net) was performed with SplitsTree4 v. 4.13.1 (Huson & Bryant, 2006) based on the Cavalli-Sforza and Edward's chord distance obtained from GENDIST in PHYLIP v. 3.695 (Felsenstein, 1989).

RESULTS

Allelic variation at microsatellite loci

All twelve microsatellite loci assayed were polymorphic, and the number of alleles detected for each locus varied between 3 (FAN30) to 20 (Sasa500) leading to 94 alleles observed in total with 2.7 % missing genotypes. These selected loci on average generated 8 alleles per locus and an expected heterozygosity of 0.708. Table 2 summarises the genetic diversity of the 12 microsatellite loci.

Genetic variation within populations

Despite the small number of plants available within populations (3 – 5 individuals), measures of average genetic diversity were relatively high (Table 3). For individual populations, the average per-locus number of alleles, rarefied allelic richness, and effective number of alleles were N_A = 4.08 (range 3.33 – 4.75), A_R = 3.75 (3.09 – 4.32) and N_{Ae} = 3.67 (2.74 – 4.25), respectively. Populations 1 (LA120) and 6 (LA159) consistently had the lowest levels of allelic diversity, while values for populations 2 (LA124) and 5 (LA 154) were consistently the highest. Similarly, for each population, the average

observed heterozygosity (H_O) and the average expected heterozygosity corrected for sample size (H_e) were 0.758 (range 0.633 – 0.834) and 0.708 (range 0.533 – 0.712), respectively. Except for the population at Adams Peak (LA159), all the other populations showed deficits in heterozygotes (average F_{IS} = 0.170; range 0.175 – 0.012). However, the LA159 population showed an excess of heterozygotes [F_{IS} = - 0.024 (Table 3)].

Table 2: Locus-level and average measures of genetic diversity for 12 microsatellite loci genotyped across six *K. debilis* populations

Locus	Sample size	Percentage of missing genotypes	N_A	N_{Ae}	H_e	H_O
Sasa500	28	0	20	7.71	0.871	0.678
Sasa718	28	0	7	5.40	0.815	0.900
Sasa11E	28	0	4	3.15	0.683	0.967
FAN11	28	3.6	6	4.95	0.798	0.845
FAN16	28	7.1	2	1.88	0.468	0.567
FAN20	28	0	7	3.33	0.700	0.678
FAN21	28	0	10	5.99	0.833	1.000
FAN26	28	0	9	4.11	0.757	0.870
FAN27	28	21.4	7	3.49	0.714	0.767
FAN28	28	0	7	4.60	0.783	1.000
FAN29	28	0	12	4.86	0.794	0.588
FAN30	28	0	3	1.40	0.284	0.245
Average over all loci	28	2.7	7.83	4.24	0.708	0.758

N_A: number of alleles with non-zero frequency; N_{Ae}: effective number of alleles; H_e: expected heterozygosity corrected for sample size; H_O: observed heterozygosity

Table 3: Measures of genetic diversity for each of the six populations of *K. debilis* averaged across 12 microsatellite loci

Population	Locality	Sample size	Percentage of missing genotypes	N_A	N_{Ae}	A_R	H_e	H_O	F_{IS}
Population 1 (LA120)	Piduruthalagala Mountain	5	1.7	3.33	2.74	3.09	0.533	0.633	0.175
Population 2 (LA124)	Horton Plains (north)	5	6.7	4.75	4.25	4.32	0.695	0.767	0.152
Population 3 (LA130)	Horton Plains (south)	5	1.7	4.50	3.81	3.93	0.632	0.750	0.042
Population 4 (LA148)	Handapan Ella Plains	3	0	3.83	4.01	3.83	0.712	0.834	0.119
Population 5 (LA154)	Handapan Ella Plains	5	1.7	4.67	4.25	4.17	0.665	0.800	0.012
Population 6 (LA159)	Adams Peak	5	3.3	3.42	2.94	3.18	0.570	0.767	- 0.024
Multilocus Average: all populations combined		28	2.7	7.83	4.24	4.58	0.708	0.758	0.170

N_A: number of alleles with non-zero frequency; N_{Ae}: effective number of alleles; A_R: rarefied allelic richness; H_e: expected heterozygosity corrected for sample size; H_O: observed heterozygosity; F_{IS}: inbreeding coefficient

Population genetic structure

The proportion of the observed genetic variation between clusters ranged from F_{ST} = - 0.053 for locus FAN27 to 0.394 for locus FAN30 with an average value of 0.113 that was significantly greater than zero ($p < 0.001$; Table 4). Individual locus estimates of the inbreeding coefficient (F_{IS}) ranged from - 0.055 (FAN26) to 0.414 (FAN29) with a mean of 0.078 ($p < 0.001$; Table 4).

Structure analyses using the Evanno method in Structure Harvester grouped the six populations into K = 3 clusters (Figures 2 and 3A). The estimated population structure inferred for K = 2 – 5 has been shown in Figure 3A. The three clusters correspond well to the geographic distribution of the populations (Figure 1), with populations 1, 2 and 3 (LA120, LA124 and LA130) forming an Eastern cluster, populations 4 and 5 (LA148 and LA154) a Southern cluster, and population 6 (LA159) a Western cluster (Figure 3A). The populations in the Eastern cluster were sampled from the Piduruthalagala Mountain (LA120) and the Horton Plains (LA124 and LA130). These two localities are in relatively close proximity and are separated by ca. 15 km. The two populations in the Southern cluster are from Handapan Ella Plains and Western cluster is from Adams Peak.

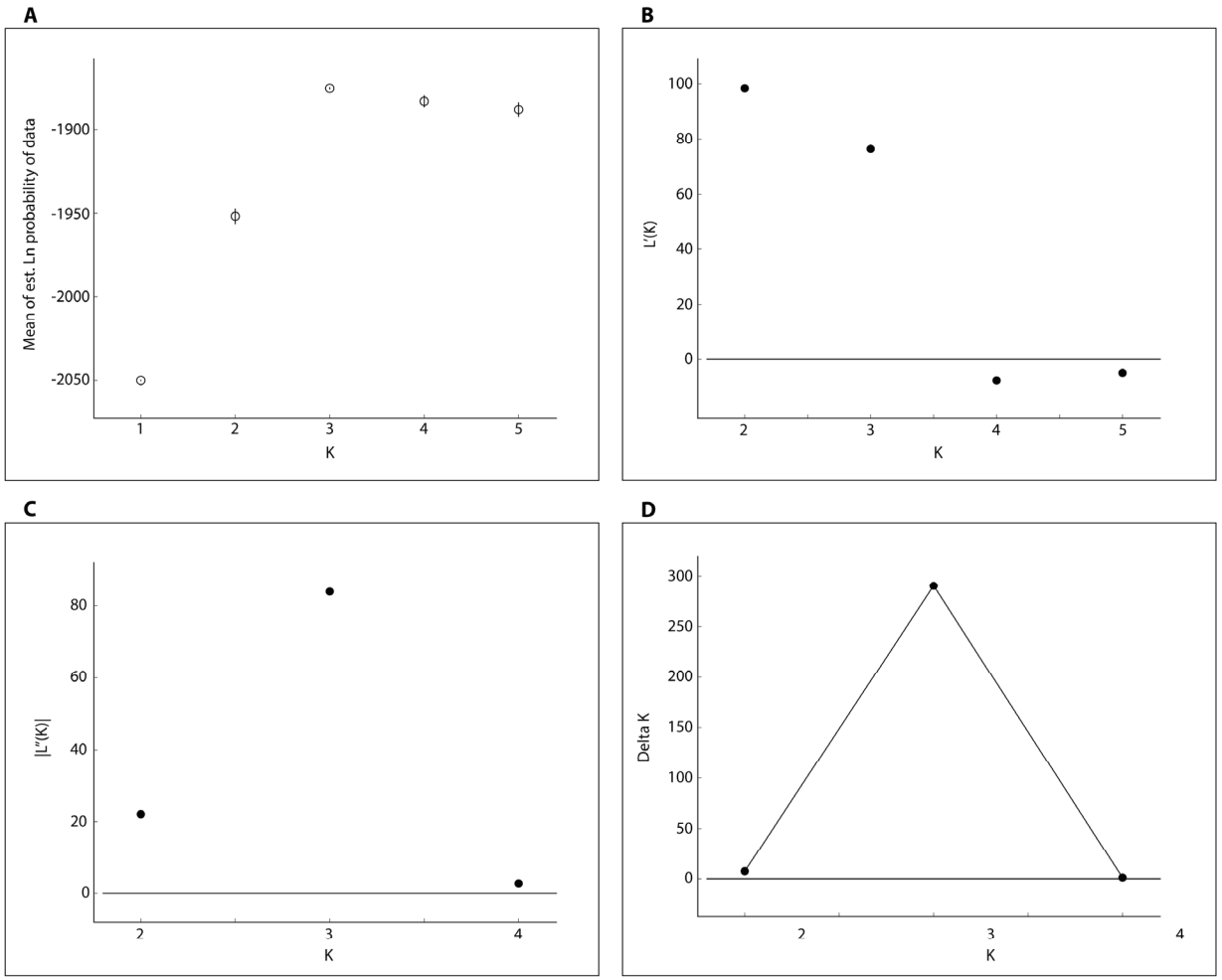

Figure 2: Structure Harvester results of structure analyses for K = 2 – 5 putative genetic clusters of *K. debilis* individuals. A: mean L(K) (± SD) over 20 runs for each K value; B: rate of change of the likelihood distribution (mean ± SD) calculated as L′(K) = L(K) – L(K– 1); C: absolute values of the second order rate of change of the likelihood distribution (mean ± SD) calculated according to the formula: |L″(K)| = |L′(K + 1) –L′(K)|; D: ΔK calculated as ΔK = mean |L″(K)|/sd[L(K)]. The modal value of this distribution is the most probable number of clusters or the uppermost level of structure, here three clusters.

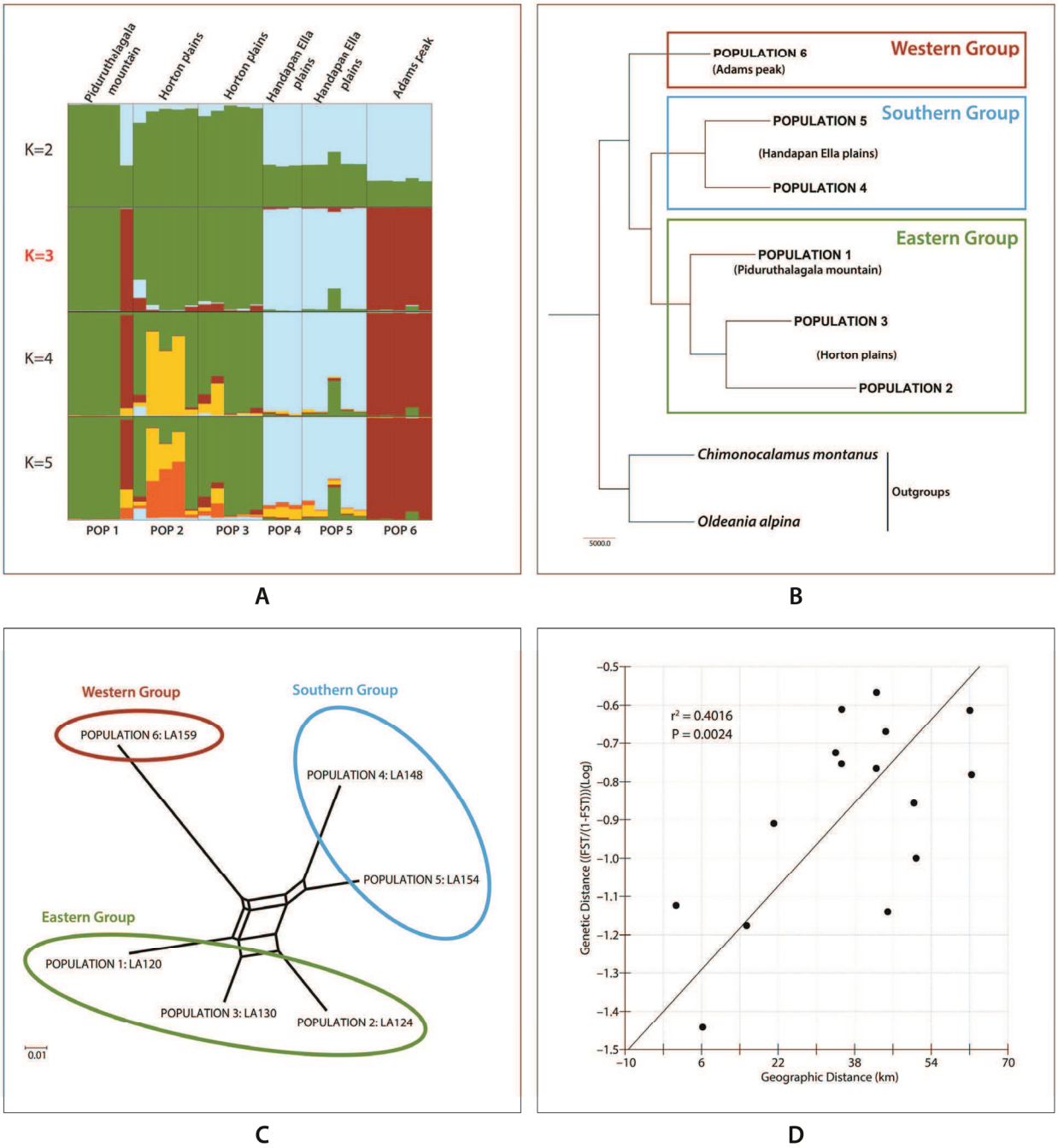

Figure 3: Analyses of genetic structure among the six *K. debilis* populations; A: Bayesian clustering using Structure for K = 2 – 5 putative genetic clusters. Each individual is represented by a vertical column and the populations are separated by a vertical black line. Different colours in the same column for each individual indicate the percentage of estimated membership in a cluster; B: rooted neighbour-joining (NJ) tree based on Cavalli-Sforza and Edwards' chord distance; C: neighbour-net network showing genetic relatedness among the study populations based on Cavalli-Sforza and Edward's chord distance; D: isolation by distance plot of Rousset's genetic differentiation on geographical distance (km)

Genetic structure inferred from AMOVA and genetic distances

Partitioning of genetic variability by AMOVA revealed that 8.35 and 7.52 % of the total genetic variation was distributed among the K = 3 clusters recognised by Structure analysis, and among populations within these clusters, respectively. Both of these values were significantly greater than zero ($p < 0.001$; Table 5). The remaining ca. 84 % of the variation was distributed among individuals within populations.

The Mantel test of the correlation between Rousset's genetic distance and geographic distance indicated strong and highly significant isolation by distance among the six *K. debilis* populations ($r^2 = 0.4016$; $p = 0.0024$) (Figure 3D).

Genetic relationships within the geographic groups

The rooted NJ tree for the six *K. debilis* populations (Figure 3B) resulted in three clades corresponding to the three genetic clusters indicated by the Structure analysis (Figure 3A). The neighbour-net network derived from the SplitsTree analysis also revealed the same three population genetic clusters (Figure 3C).

DISCUSSION

Locus and population level genetic diversity

Of the six *K. debilis* populations sampled for this study, population 1 (LA120), which was collected from mount Piduruthalagala was unusual in displaying lower allelic and genetic diversity compared to the other five populations (Table 3). The lower diversity of this population is not due to a smaller sample size or more missing loci, as these were similar to the other populations examined. The 2.7 % of missing genotypes is due to failure to amplify certain loci.

Of the various dimensions of genetic diversity, allelic richness is often considered to be of key relevance in conservation programmes (Petit *et al*., 1998; Simianer, 2005; Foulley & Ollivier, 2006). Allelic diversity is particularly sensitive to bottlenecks in population size and genetic drift, and may be an important indicator of a population's adaptive potential, as the limit of selection response is mainly determined by the initial number of alleles regardless of the allelic frequencies (Hill & Rasbash, 1986). Based on these expectations, Petit *et al*. (1998) regarded allelic richness as the most informative measure of genetic variation for identifying populations for conservation. From this perspective, population 1 may be of lesser value for conservation than populations 2 – 6.

Table 4: Individual locus and average F-statistic measures estimated across six populations of *K. debilis*

Locus	F_{IT}	F_{IS}	F_{ST}
Sasa500	0.417***	0.251***	0.221***
Sasa718	-0.012	-0.099**	0.079**
Sasa11E	-0.039	-0.103	0.058*
FAN11	0.148***	-0.006	0.153***
FAN16	0.250**	0.163	0.103
FAN20	0.377***	0.312***	0.095*
FAN21	-0.009	-0.112**	0.092***
FAN26	0.082	-0.055	0.130***
FAN27	0.095	0.140*	-0.053
FAN28	0.043	-0.013	0.055*
FAN29	0.508***	0.414***	0.161
FAN30	0.531***	0.225**	0.394***
Average	**0.183*****	**0.078*****	**0.113*****

F_{IT}: fixation index as the global population; F_{IS}: inbreeding coefficient in relation to subpopulations; F_{ST}: inbreeding due to differentiation of subpopulations in the total population. Significance of average F-statistic estimates: *, **, and *** denote p values less than 0.05, 0.01, and 0.001, respectively.

Table 5: AMOVA analysis of genetic differentiation among the K = 3 clusters recognised by Structure analysis and among populations within these clusters

Source of variation	df	SSD	Variance components	Percentage of variance	Φ_{ST}	Φ_{SC}	Φ_{CT}
Among clusters	2	38.159	0.314	8.35			
Among populations within clusters	3	25.069	0.283	7.52			
Within populations	106	335.817	3.168	84.13			
Total	111	399.045	3.765		0.159*	0.082***	0.084***

Significance of estimated phi-statistics (see text): * and *** denote p values less than 0.05 and 0.001, respectively.

Vekemans and Hardy (2004) surveyed fine-scale spatial genetic structure analyses in plant populations based on both allozymes and microsatellites and showed that, on average outcrossing species have a F_{IS} value of 0.014. The global F_{IS} value of 0.078 (Table 4) obtained for *K. debilis* is significantly low and indicates that these populations are primarily outcrossing.

Genetic structure of *K. debilis* populations

Genetic differentiation among angiosperm populations arises due to a variety of factors. Factors associated with relatively low values of genetic differentiation include woody habit, outcrossing breeding systems and wind dispersal (Hamrick *et al*., 1992), all of which are characteristic of *K. debilis*. Based on Wright's (1978) qualitative guidelines for the interpretation of F_{ST} , the global value for *K. debilis* (F_{ST} = 0.113) obtained in this study is within the suggested range for moderate genetic differentiation (range: 0.05 – 0.15). That said, it is rather high given the relatively close spatial proximity of the study populations. This study showed that *K. debilis* populations cluster into three genetically distinct sub-groups in the central mountains of Sri Lanka: one specific to the East (populations LA120, LA124 and LA130), one specific to the South (populations LA148 and LA154) and a single unique population to the West (LA159). These groupings were evident from all three of the clustering analyses used (structure, neighbour joining, and neighbour-net). The observation that the number of genetic clusters being lower than the number of populations is indicative of partial barriers to gene flow, which may be either historical or ongoing (Vergara *et al*., 2014). Also consistent with this observation, the Mantel test shows that geographic distance explains 40 % of the genetic distance between population pairs. This positive association between geographic distance and genetic distance suggests that migration rates between these *K. debilis* populations decrease with increasing distance as expected under an isolation by distance (IBD) model of gene flow (Wright, 1943).

Conservation implications

This analysis is the first study investigating the genetic diversity and structure of *K. debilis* from Sri Lanka, with samples from almost all known populations evaluated using microsatellite markers. Although several studies have previously quantified population level genetic variation in bamboos, none of them have related this variation to the conservation of bamboo populations or species (Ramanayake *et al*., 2007; Lalhruaitluanga & Prasad, 2009; Mukherjee *et al*., 2010; Triplett & Clark, 2010). Populations 2 – 5 showed generally high levels of genetic diversity (Table 4). Population 2 (LA124) from the northern part of the Horton Plains showed the highest levels of N_A, N_{Ae} and A_R, and is a particularly good candidate for conservation. The single population from Adams Peak (population 6: LA159) formed its own genetically distinct cluster and is also a potential candidate for conservation due to its unique genetic diversity. Although the three genetic clusters do not provide evidence for morphologically distinct ecotypes, their unique genetic diversity must be taken into consideration by conservation managers when identifying conservation management units. Many studies show that, while ecotypes do matter when defining management units, recommendations for conservation are commonly made based on the genetic uniqueness of the populations (Soule & Simberloff, 1986; Barrett & Kohn, 1991). As the six populations of *K. debilis* formed three genetically identifiable clusters, we recommend that populations separated by more than ca. 35 km (as is the case for these clusters) should be treated as distinct units for management and conservation, while those within ca. 15 km should be managed jointly. These recommendations serve as an initial step towards identifying management units for threatened bamboos in Sri Lanka. However, since our sample sizes are relatively smaller one must use these results cautiously.

Despite the small sample sizes, levels of genetic variation were found to be relatively high within individual populations. Further, tests of genetic differentiation among populations were found to be highly significant. Consequently, this study provides important insight into the genetic diversity and connectivity of *K. debilis* and is a significant initial step towards the conservation management of this threatened temperate woody bamboo species. For these rare and threatened species with limited distribution areas, a single stochastic event, such as a serious insect attack or pathogen infection, could cause catastrophic reductions in population size and genetic diversity, and even extinction (Ma *et al*., 2013). Taking proper measures to protect their current populations is required.

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