

Genetic diversity and structure of *Ochlandra travancorica* populations from Kerala part of the Western Ghats

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Abstract: *Ochlandra travancorica* is one of the economically important endemic reed bamboos of the Western Ghats. They are indiscriminately harvested for commercial usage in paper, pulp, and traditional cottage industries. This has drastically affected its viable populations in the Western Ghats. SSR markers were employed for characterizing the selected natural population to assess genetic diversity and infer genetic structure in their natural distribution range. The marker analysis revealed existence of high genetic diversity in sampled populations of *O. travancorica* ($H_e=0.834$, $I=2.092$). Analysis of molecular variance (AMOVA) revealed that a large proportion of genetic variation (84 %) is confined within the populations and only 16 percent was observed between populations. The pattern of genetic admixture generated in STRUCTURE analysis revealed high level of substructuring of populations. This might be due to close proximities of populations and high amount of gene flow ($Nm=1.456$) among them. Genetically diverse populations as indicated by number of, private alleles, gene diversity, heterozygosity and polymorphic content in different geographical areas indicate the need for *ex situ* conservation and genetic improvement programmes. Periyar and Malayattoor populations with significant genetic admixtures can also be recommended for resource conservation of reed bamboo species.

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Introduction

Bamboo constitutes an important group of plants having vital economic as well ecological importance. Bamboo has a multipurpose utility which plays a crucial role in culture, art, industries, and construction due to their peculiar nature like thin-walled, thickly clumped culms (Kumar, 2011; Seethalakshmi and Kumar, 1998). In Kerala, bamboo cottage industries mainly use reed bamboos, particularly *O. travancorica* and support the livelihood of the economically weaker strata of the society (Kumar, 1988). Worldwide, there are 1700 species of bamboo distributed among 127 genera, three tribes, and 15 subtribes (Li *et al.*, 2021). In India, 148 bamboo species comprising 29 genera are distributed in the Western Ghats, North-east India, Andaman-Nicobar Islands, and is the second largest reserve of bamboo resources in the world (Sharma and Nirmala, 2015). The Western Ghats with 22 species in seven genera, constitute a major component of bamboo diversity (Kumar, 2011) and microspot for endemic bamboos (Mittermeier *et al.*, 2005).

Usually bamboo species show characteristic flowering and fruiting cycles, ranging from a few years to 120 years (Janzen, 1976). *O. travancorica* exhibits both gregarious and sporadic flowering behaviors (Koshy and Harikumar, 2001) which often lead to the decline of the huge reed population. Their natural regeneration usually occurs through seeds and rhizomes. The matured clumps are formed within a period of 6-8

years (Seethalakshmi and Kumar, 1998). The presence of dichogamous spikelets increases pollination rate in *Ochlandra* species (Venkatesh, 1989). This species is widely distributed in Kerala part of the Western Ghats and had sparse distribution in Tamil Nadu and Karnataka. Reed bamboos function as a keystone species in evergreen forests by influencing the survival of many associated species and their ecological niches (Basha, 1991).

Although reed bamboos have been heavily exploited for paper and pulp industries over years, resulting in

severe depletion of natural resources, no stringent scientific management practices have been implemented except some policy interventions (seasonal ban on extraction) to manage the sustainable use of reed resources (Nair, 1991). Currently, reed bamboos are legally protected in Wildlife Sanctuaries, but continuous extractions are still happening from territorial divisions. Sustainable management and conservation of available resources necessitate an understanding of genetic diversity and structure in populations of reed bamboos in the Western Ghats.

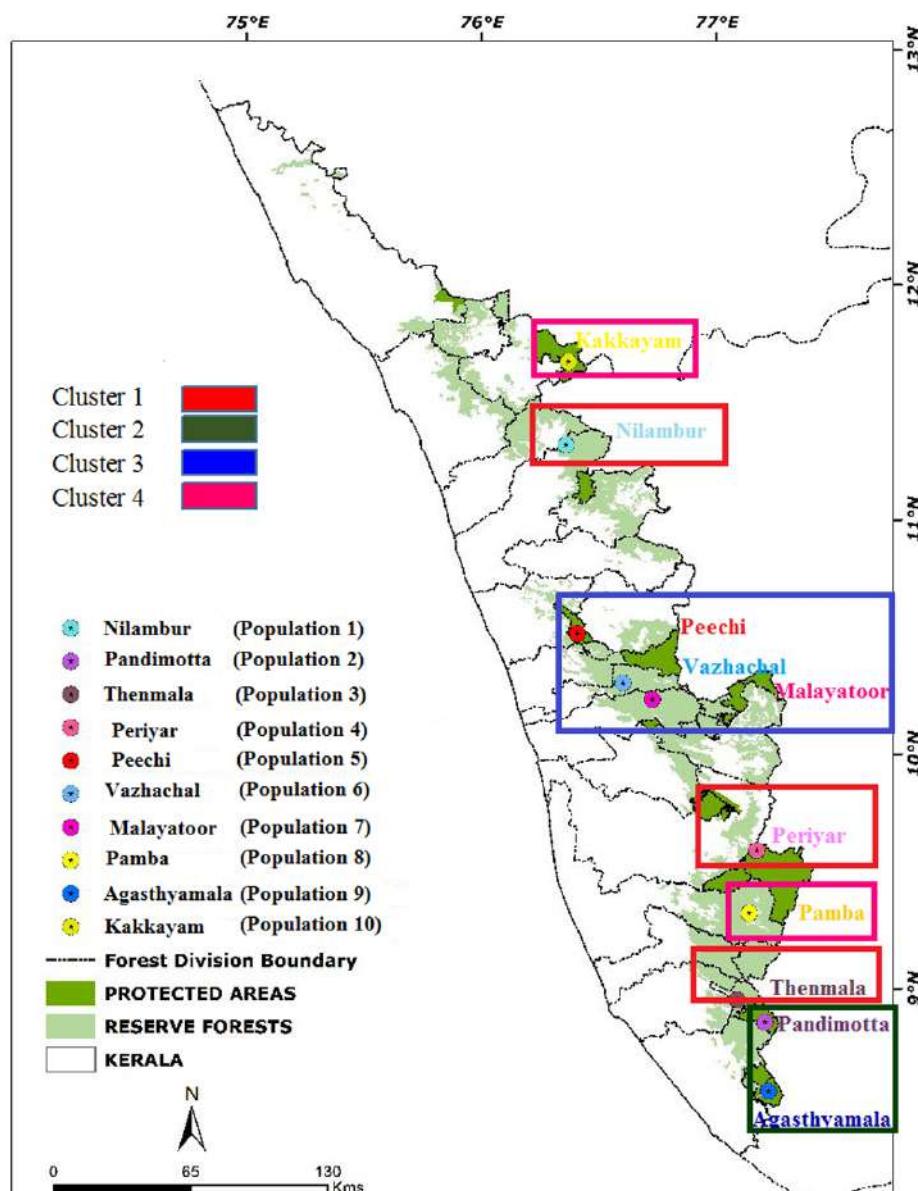


Fig. 1. Sampled populations of *O. travancorica* based on genetic clusters in the Kerala part of Western Ghats

Studies on genetic diversity of three *O. travancorica* populations using dominant (AFLP and RAPD) markers reported high level of genetic variation within populations and suggested the necessity for further assessment using more accessions (Nag *et al.*, 2013). ‘Microsatellites’ or simple sequence repeats (SSR) are one of the highly versatile genetic markers which is distributed throughout the genome (Litt and Luty, 1989; Jiang *et al.*, 2014, Nilkantha *et al.*, 2017). Several studies using SSR markers were earlier reported in bamboo species (Yasodha *et al.*, 2010, Jiang *et al.*, 2014). Cross transferability of SSR and EST-SSR markers was reported in *Dendrocalamus latiflorus* (Bhandawat *et al.*, 2014), *Phyllostachys pubescens* (Lin *et al.*, 2014) and *Arundinaria alpina* (Disasa *et al.*, 2018). Genome-wide SSR markers were successfully validated in moso bamboo (*Phyllostachys edulis*; Zhao *et al.*, 2015).

As there is limited information on the genetic diversity and population structuring of endemic *O. travancorica* populations in the Western Ghats the present study analyzes population genetic diversity pattern and structuring of selected ten natural populations of *O. travancorica* from Kerala.

Materials and methods

Site of collection

A. Plant materials

Ten populations of *O. travancorica* in the natural distribution zones of Kerala, both in the protected (Wildlife sanctuaries, National Park) and territorial/extracted areas, were selected for the study (Fig. 1). Fresh leaves were collected from fifteen different clumps in each population and stored in silica gel. Geographic locations and GPS coordinates of studied populations are presented in Table 1.

Table 1. Sampled wild populations of *Ochlandra travancorica* from the Kerala part of Western Ghats

Population	Geographic regions	GPS coordinates	Protected area/ Extracted area
Nilambur (Pop 1)	Nilambur North and South	11°19'02.8"N 76°21'31.4"E	Extracted area
Pandimotta (Pop 2)	Shendurney WLS	8°51'24.1"N 77°12'20.4"E	Protected Area
Thenmala (Pop 3)	Aryankavu, Achenkovil	8°57'10.2"N 77°05'12.6"E	Extracted area
Periyar (Pop 4)	Thekkady WLS	9°35'18.8"N 77°10'16.4"E	Protected Area
Peechi (Pop 5)	Peechi-Vazhani WLS	10°30'46.8"N 76°24'23.2"E	Protected Area
Vazhachal (Pop 6)	Athirapally	10°18'08.2"N 76°36'02.4"E	Extracted area
Malayatoor (Pop 7)	Malayatoor, Idamalayar	10°13'54.7"N 76°43'32.9"E	Extracted area
Pamba Kakki (Pop 8)	Pamba Kakki Gudrickal	9°19'17.3"N 77°08'18.4"E	Extracted area
Agasthyamala (Pop 9)	Neyyar WLS	8°33'43.8"N 77°13'14.6"E	Protected Area
Kakkayam (Pop 10)	Malabar WLS	11°40'17.6"N 76°22'07.4"E	Protected Area

B. Genomic DNA extraction

Total genomic DNA was extracted using modified Cetyl trimethyl ammonium bromide (CTAB) method from 20 mg leaf samples (Doyle and Doyle, 1987). Quantitative and qualitative analyses of genomic DNA was performed using NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific, UK) and visualized in 1.0 per cent agarose gel under ultraviolet-light (UVP BioImaging Systems, Upland, CA).

C. PCR amplification and SSR genotyping

Ten SSR primers were initially selected from *Dendrocalamus latiflorus* (Bhandawat et al., 2014). The forward primers were labelled with fluorescent dye (6-FAM) for genotyping. PCR amplification was performed in a PTC-100 thermal cycler (BIO-RAD, India) with 20 µL reaction mixture, containing 50-100 ng DNA, 10X Taq buffer with 1.5 mM MgCl₂, 200 µM dNTPs, 20 pm of each primer and 2U Taq DNA polymerase (Invitrogen, Bengaluru). PCR cycling conditions included an initial denaturation at

94°C for 10 min; then 30 cycles of denaturation at 94°C for 50 sec, annealing temperature (50°C-60°C) for 1 min depending upon primers, and extension at 72°C for 1 min; and a final extension at 72°C for 15 min. (Table 2). PCR products were resolved in 2 per cent agarose gel and visualized using a gel documentation system (Syngene, UK). Genotyping of SSR fragments were performed using ABI 3730XL capillary automated sequencer (Applied Biosystems) with an internal size standard (Gene Scan-500 LIZ, Applied Biosystems) at AgriGenome Labs (Cochin, Kerala).

Marker Data Analysis

A. SSR polymorphism and genetic diversity

Basic statistics such as major allele frequency, gene diversity and polymorphic information content (PIC) were determined for polymorphism detection using PowerMarker v.3.25 (Liu and Muse, 2006). The parameters to assess the genetic diversity among ten populations viz. the number of alleles (Na), effective number of alleles (Ne), number of

Table 2. Microsatellite marker details used for PCR amplification in *Ochlandra travancorica*

Locus	Repeat motif	Primer sequence (5'-3') 5'- 6-FAM labeled forward primer	Annealing Temperature (°C)	Allele size range (bp)
SSR1	(GA) _n	TCCCATTGTCCGTCTCTTC CAGCCTCATCACCATCCTCT	55	146-219
SSR2	(CGG) _n	CTATCCTCCTCCCCAAATCC TTCGCTTCGAGGGTTAAATG	51	154-190
SSR7	(CAG) _n	ACAGAGGCCACAAGTTCCAC TGACACAGGAGTCCGAACAG	55	210-321
SSR8	(ATG) _n	AAGGAAAAAGGGCTGGGTTA TCGTCGTCATCACTTGCTC	54	179-290
SSR10	(TTC) _n	CGCAGCTACACTGCACAAGT GCAAAGATGTTCCCTGCAAT	53	406-429
SSR11	(TC) _n	GGGGGAGAAGGAAAGAGAGA GCAGGGAATAAGCAAGCAAT	57	154-200
SSR14	(GA) _n	TCTCTCCTCCCTTCAAACA ATATACCCTGCGAGCTGGTG	52	135-196

Source: Bhandawat et al., 2014

of private alleles (N_p), observed heterozygosity (H_o), expected heterozygosity (H_e) and Shannon Information Index (I) (Lewontin, 1972) were estimated using GenAIEx v.6.501 (Peakall and Smouse, 2012). The frequencies of null allele were estimated using the Micro-Checker v.2.2.3 package (Van Oosterhout *et al.*, 2004).

B. Population structure and gene flow

The components of variances among populations as well as at individual level was estimated using Analysis of Molecular Variance Analysis (AMOVA). Population genetic differentiation by means of fixation index (F_{ST}) over populations, inbreeding coefficient with respect to subpopulations (F_{IS}), pairwise F_{ST} between populations, and gene flow (Nm) was assessed in GenAIEx v.6.501 (Peakall and Smouse, 2012). Gene flow among populations was estimated based on the number of migrants per generation (Nm) (Slatkin and Barton, 1989). Bayesian model-based clustering algorithm was used to infer number and pattern of subpopulations and detection

of admixtures among populations implemented in STRUCTURE v.2.3.4 (Pritchard *et al.*, 2000). Simulations were run ten times for each value of K (2-15) with 10^5 Markov Chain Monte Carlo (MCMC) generations after a burn-in period of 10^5 iterations. Optimum K value was determined using graphical method of Evanno *et al.*, (2005) based on change of the likelihood function with respect to K in Structure Harvester v. 6.92 (Earl and Von Holdt, 2012). Output files of 25 iterations at K 2-15 were run in CLUMPP v.1.1.2 (Kopelman *et al.*, 2015) and visualized using DISTRUCT v.1.1 (Rosenberg, 2004).

Results

SSR polymorphism and population genetic diversity

Out of ten SSR markers, seven were highly informative with Polymorphic Information Content (PIC) ≥ 0.7 i.e., varied from 0.7781 to 0.9674 (Fig. 2;Table 3). Genetic diversity indices of the ten analyzed populations are summarized in Table 4. The average number of alleles over loci per population ranged from 7.000 to 10.571.

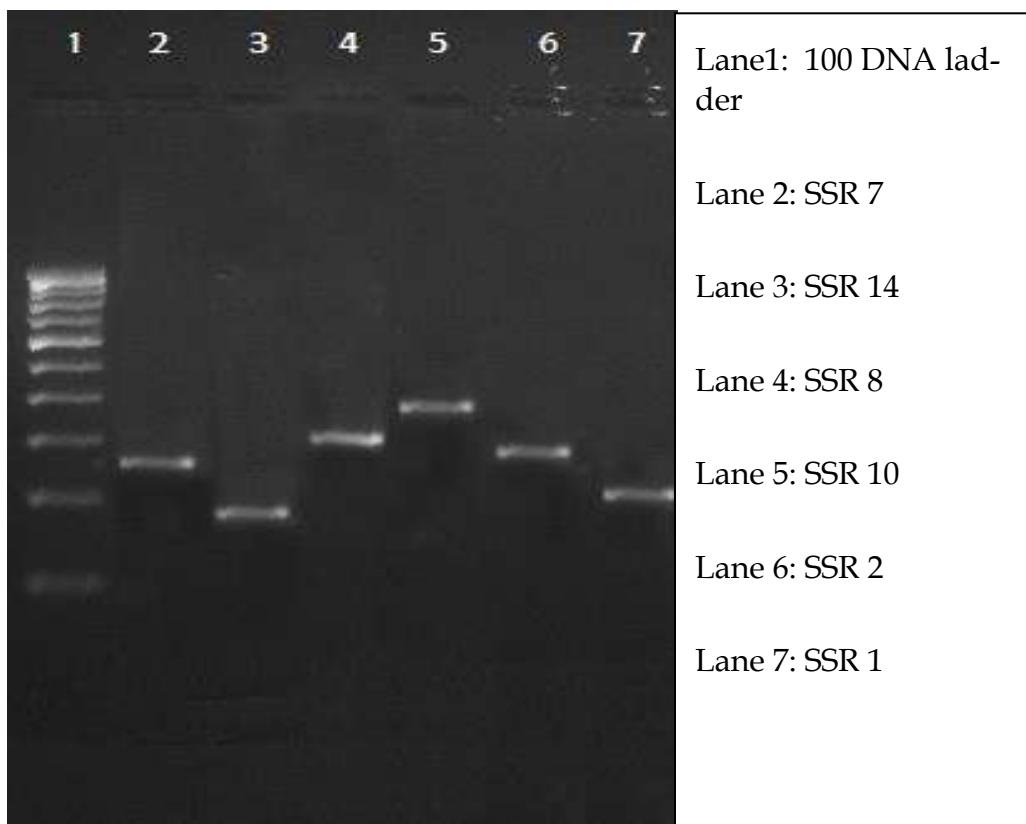


Fig. 2. SSR amplified products on agarose gel

Table 3. Measures of genetic polymorphism for SSR markers across ten populations of *O. travancorica*

Microsatellite locus	MAF	GD	PIC
SSR1	0.3333	0.8451	0.8346
SSR2	0.1708	0.8994	0.8913
SSR7	0.1000	0.9676	0.9667
SSR8	0.1208	0.9682	0.9674
SSR10	0.3583	0.7998	0.7781
SSR11	0.2292	0.9026	0.8963
SSR14	0.2000	0.9223	0.9183

MAF = Major allele frequency; GD = Gene Diversity; PIC = Polymorphic Information Content

Besides, private alleles varied from 10 to 25 were present in all the studied populations. The frequencies of null allele estimates obtained using different models are provided (Table 5). Although null allele is present in a single locus (SSR 7), there were no statistical differences observed between allele frequencies of that locus among the models ($p > .05$) and hence included in subsequent analyses. Expected heterozygosity was highest in Periyar (Pop

4; $H_e=0.834$) and lowest in Pandimotta (Pop 2; $H_e=0.682$). Similarly, observed heterozygosity was highest in Kakkayam (Pop 10; $H_o=0.821$) and lowest in Malayatoor (Pop 7; $H_o=0.583$). The mean genetic diversity (Shannon's Diversity Index, I) estimated was greater than 1 in all populations with an average of 1.777. The highest was observed in Periyar Tiger Reserve (Pop 4; $I=2.092$) and lowest in Pandimotta (Pop 2; $I=1.580$) populations (Table 4).

Table 4. Population diversity indices among ten populations of *O. travancorica*

Populations	Na	Ne	Np	H _o	H _e	I	F
Nilambur (Pop 1)	7.714	5.467	14	0.619	0.724	1.647	0.160
Pandimotta (Pop 2)	7.714	5.072	15	0.607	0.682	1.580	0.131
Thenmala (Pop 3)	10.571	7.478	18	0.726	0.750	1.888	0.009
Periyar (Pop 4)	10.571	7.601	15	0.774	0.834	2.092	0.074
Peechi (Pop 5)	7.000	4.603	10	0.690	0.729	1.588	0.098
Vazhachal (Pop 6)	9.143	6.170	13	0.702	0.803	1.919	0.134
Malayatoor (Pop 7)	8.429	5.417	10	0.583	0.776	1.789	0.266
Pamba (Pop 8)	8.143	5.373	19	0.702	0.765	1.749	0.085
Agasthyamala (Pop 9)	8.571	5.295	25	0.643	0.744	1.733	0.126
Kakkayam (Pop 10)	8.714	5.674	18	0.821	0.768	1.790	0.093
Mean	8.657	5.815		0.687	0.757	1.777	0.099

Na, number of different alleles; Ne, number of effective alleles; Np, number of private alleles, H_o, observed heterozygosity; H_e, expected heterozygosity; I, Shannon's information index; F, fixation index

Table 5. Summary statistics for the estimation of null allele in Microchecker v.2.2.3

Locus	Null present	Oosterhout	Chakraborty	Brookfield1	Brookfield2
SSR1	No	-0.0879	-0.0627	-0.0579	0.1023
SSR2	No	0.0871	-0.0843	-0.0843	0.0325
SSR7	Yes	0.0943	0.1081	0.0931	0.1976
SSR8	No	0.0187	0.0176	0.0164	0.1298
SSR10	No	0.0012	-0.0042	-0.0037	0.1338
SSR11	No	-0.1917	-0.1155	-0.1111	0.0787
SSR14	No	0.0051	0.0076	0.0071	0.1253

Genetic differentiation and Population genetic structure

Genetic differentiation among populations was measured by Wright's Fixation Index (F_{ST}) and the pairwise F_{ST} estimated ranged from 0.046 to 0.105 with an average of 0.162. F_{ST} revealed 16 % of variation among populations and remaining 84 % within population. This is consistent with results of AMOVA which showed 15 % variation among populations. The inbreeding coefficient (F_{IS}) varied from -0.139 to 0.199 with an average of 0.095 over populations. Gene flow among populations (Nm) was observed to be moderately high (1.456) (Table 5).

For genetic structuring, $\text{LnP}(D)$ was calculated for all K values in each simulation model and K value with

maximum $\text{LnP}(D)$ is considered as optimum number of subdivisions. Evanno *et al.*, (2005) method on structure output predicted K with clear peaks for $K=4$ and 6 with negligible difference in ΔK values of both (Fig. 3). A linear graph was observed in log likelihood [$\text{LnP}(D)$] against K values with a slight decrease at $K=6$ (Fig. 3). Hence, output data in CLUMPP with maximum probable values of ΔK inferred as 4 likely number of clusters/subpopulations was visualized in DISTRUCT (Fig. 4). Principle Coordinate Analysis (PCoA) based on genetic distance also was congruent with CLUMPP results (Fig. 5). Genetic structuring analysis assigned ten populations into four clusters with genetic similarity in some populations and admixtures in other.

Table 6. Analysis of molecular variance (AMOVA) and F statistics measures estimated at population level

Source of variation	Degrees of freedom	Estimated variance	% of variation	Genetic differentiation*	Gene flow
Among population	9	0.428	13	$F_{IS}=0.095$	
Among Individuals	110	0.379	12	$F_{IT}=0.241$ $F_{ST}=0.162$	$Nm=1.44$
Within individual	120	2.404	75		

F_{IS} = Inbreeding coefficient with respect to subpopulations; F_{ST} = Inbreeding due to differentiation of subpopulations within total populations; F_{IT} = Fixation index as global population; Nm = estimate of gene flow

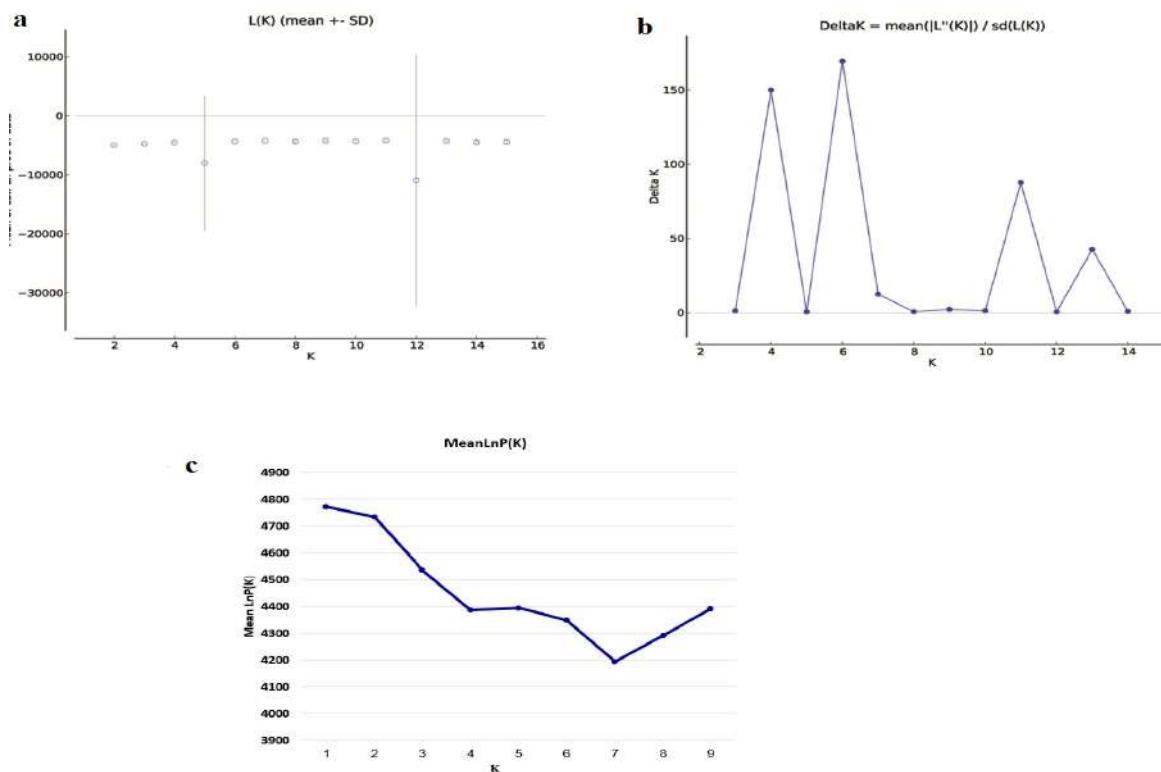


Fig. 3. Results of the Bayesian model based clustering analysis in STRUCTURE.; (a) The probability of the data in P(D) (\pm SD) against K clusters.; (b) ΔK values from the mean likelihood probabilities from runs where inferred clusters (K) ranged from 2 to 15.; (c): the average log-likelihood of K-value against the number of K

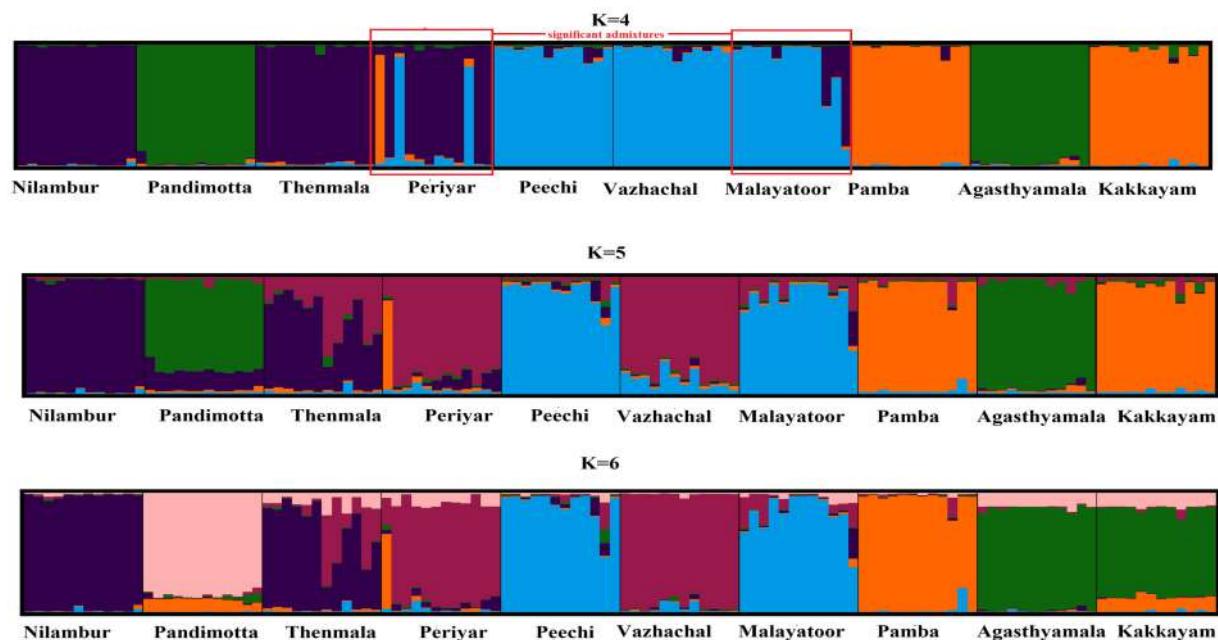


Fig. 4. Population genetic structure pattern of ten populations of *O. travancorica* using DISTRUCT K = 4 to 6

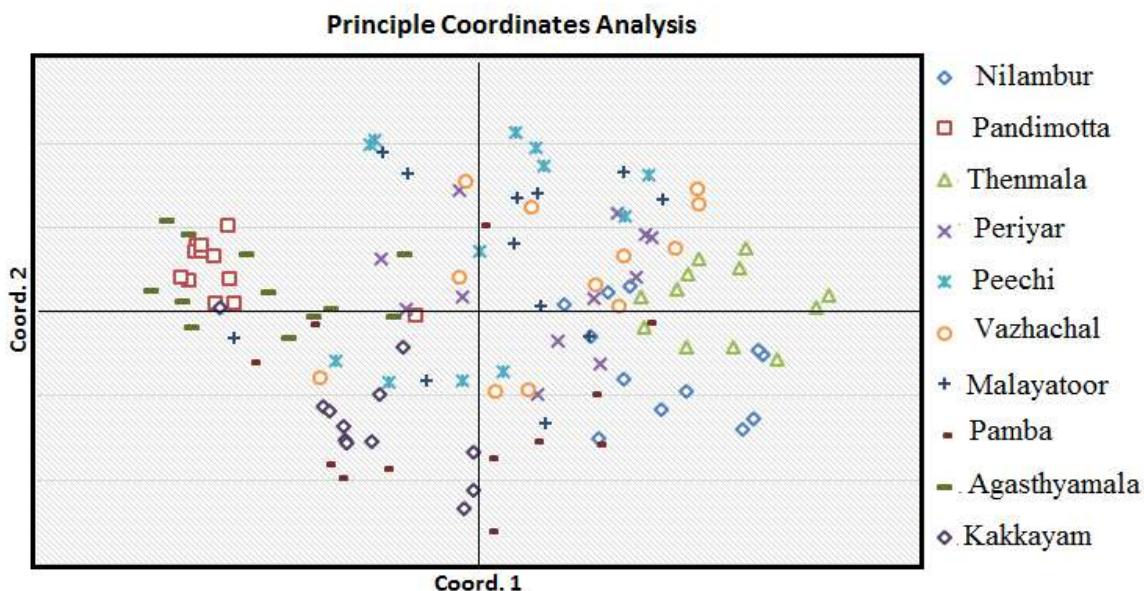


Fig. 5. Principle Coordinate Analysis (PCoA) showing clustering of individuals of ten populations of *O. travancorica*

Discussion

Genetic Diversity

Genetic diversity assessment and structuring pattern of *O. travancorica* revealed high genetic diversity within populations and low genetic differentiation among populations. Polymorphic information content (PIC) of a marker reveals its usefulness in the diversity analysis and a PIC value of 0.5 is considered as highly informative and 0.25 to 0.5 as moderately informative and <0.25 as least informative (Zuo *et al.*, 2019). A minimum PIC value of 0.8 observed in the populations indicates their abundant genetic diversity. Similarly, the deviation from Hardy-Weinberg equilibrium (HWE) ($H_o < H_e$) was observed in the studied populations except for Kakkayam, which might be due to the biparental inbreeding Plant species having a wide range of geographical distribution also possess a higher level of genetic diversity than isolated populations (Chen *et al.*, 2014). Periyar population has a wide area of species distribution and is situated in a highly protected zone with limited anthropogenic influences. Shannon's diversity index accordingly, was highest in Periyar ($I=2.092$) with high observed and expected heterozygosities ($H_o=0.774$; $H_e=0.834$). Seethalakshmi and Kumar (1998) reported wide distribution of *O. travancorica* particularly in Kerala part of the Western Ghats.

Allelic richness and private alleles are important indicators of genetic diversity which are key factors for selection of superior populations for effective conservation (Foulley and Ollivier, 2006). Private alleles can be considered as indicators of gene flow and are correlated with mean number of migrants exchanged per generation between populations (Barton and Slatkin, 1986). Agasthyamala (Pop 9) bear a relatively high proportion of private alleles which indicates a discrete gene pool. The population possesses high species density and regeneration, due to limited human intervention in this Biosphere Reserve located at the extreme end of reed distribution zone. The estimation of null alleles using statistical methods are based on the assumptions that the presence of null alleles produces an excess of homozygotes in the populations, in comparison with the expected Hardy-Weinberg equilibrium (Van Oosterhout *et al.*, 2004). Also, microsatellite null alleles have been found in a wide range of taxa, including species where N_e is not necessarily large. The presence of null alleles may be problematic when comparing different populations with different null allele frequencies or characterized by low levels of gene flow (Chapuis *et al.*, 2007). The present study revealed the deficit in heterozygotes in a single locus but there is no evidence for scoring due to stuttering and no large allele dropout (Table 5).

It is important to have diverse alleles in a population because the presence of new alleles enhances the ability of a species to adapt in various environmental conditions. The effective alleles (4.603 to 7.601) are less than the average number of alleles (ranged from 7.000 to 10.571), is indicative of the deviation from Hardy Weinberg equilibrium in the analyzed populations (Templeton, 1980; Young *et al.*, 1996).

Deviation from Hardy-Weinberg equilibrium could result from non-random mating within populations due to unique biological characteristics of bamboo species, such as clonal propagation, monocarpic nature or long flowering intervals (Janzen, 1976). Higher level of genetic diversity within population was reported in other bamboo species such as *Dendrocalamus membranaceus* ($H_s=0.349$) (Yang *et al.*, 2012) and *Melocanna baccifera* ($H_s=0.1639$) (Nilkantha *et al.*, 2017). Significant level of genetic diversity was also reported in widespread endemic congeneric species, *Adenophorus periens* (Ranker, 1994), *Daviesia suaveolens* (Young and Brown, 1996), *Caesalpinia echinata* (Cardoso *et al.*, 1998) and *Pinus rzedowskii* (Delgado *et al.*, 1999).

There are two probable assumptions for the observed high level of genetic diversity as well as allelic richness in the present study. Positively, it must have resulted from seed mediated natural regeneration in this self-incompatible species which favors cross breeding. However, high genetic diversity can also be found within small populations if population size has been reduced recently, especially if reduction has occurred within a generation or two (Avise, 1995; Luan *et al.*, 2006). *O. travancorica* was once evenly distributed throughout the Western Ghats and continuous unscientific reed extractions had happened in the recent past (Nair, 1991; Gopakumar and Motwani, 2013). Large tracts of bamboo forests have been wiped out in the Kerala forests consequent to reed extraction (Noushad, 2008) and these fragmentations contribute to geographic isolation and evolution of the populations as discrete units. Recently, Government of Kerala, India proposed a closure period (June to September) for reed extraction in order to facilitate regeneration of new sprouts for the sustainable management of reed bamboo resources. In spite of all these regulations, reed bamboos are under tremendous extraction pressure and immediate scientific interventions are required

to support effective regeneration and sustainable management of this endemic reed species.

Genetic differentiation and gene flow

High gene flow ($Nm=1.456$) mediated through pollen and seed dispersal indicates the close proximities of past *O. travancorica* populations in the Kerala part of Western Ghats. However, habitat fragmentation may lead to genetic changes in remnant populations and accelerate the genetic divergence among populations by means of reduced gene flow and increased random drift. Since, *O. travancorica* is an ecologically as well as economically exploited reed bamboo species, human mediated movement of genotypes across populations is also possible. High gene flow leads to a moderately low genetic differentiation among *O. travancorica* populations (16 %). According to Hamrick *et al.* (1995), gene flow estimates for con-specific populations sampled miles apart vary over species from very low ($Nm<<0.5$) to very high ($Nm>>5.0$) and this low gene flow is often evolutionarily significant enough to counteract genetic drift ($Nm>>0.5$). McDermott and McDonald (1993) indicated that if $Nm>1$, there will be little differentiation among populations and migration counter-balance the effect of genetic drift. Average inbreeding coefficient ($F_{IS}=0.095$) estimated is higher than the reported average (0.014; Vekemans and Hardy, 2004) which again is indicative of the clonal recruitment and subsequent biparental inbreeding to an extent in this genus. Clonal expansion also enhances chances of pollen transfer between flowers of same genets and may likely influence both female and male mating costs (Harder and Barrett, 1995). Clonal growth usually affects reproductive success of both male and female individuals with a high degree of clonal intermingling resulting in increased individual genet size as reported in dwarf bamboo, *Sasa veitchii* var. *hirsuta* (Matsou *et al.*, 2014).

Population genetic structure and Conservation Implications

Despite the distribution of species either in protected or extracted zones, they preserve a high amount of genetic variations over long periods. However, the genetic structuring of *O. travancorica* revealed four subpopulations/clusters (four ancestral areas) with genetic admixtures irrespective of being protected or

extracted areas (Fig. 2). The lower number of clusters than the number of populations indicates the presence of sufficient gene flow amongst populations evidenced by admixture patterns (Banaszek *et al.*, 2012). Higher genetic diversity is found in larger and older populations when compared to small and newly established ones, which is vital and an indication of their fitness (Luo *et al.*, 2019). Periyar with maximum genetic admixture is located in the reserve/protected area and genetic composition is well preserved without any human intervention or any anthropogenic activities. Significant admixtures of populations found in this protected zone (Periyar) revealed the possible ancestral origin of reed populations. High gene flow amongst populations coupled with admixtures indicate the founder effect, proposed in endemic species as originated from small parental populations during the course of past events (Mayr, 1959, Barton and Charlesworth, 1984, Carson and Templeton, 1984). Nilambur and Thenmala populations, in two different geographic zones, displayed similar genetic patterns and shared ancestry to the Periyar population. Pandimotta and Agasthyamala forest areas are situated in geographic proximity to well-preserved gene pool of similar genetic composition without any significant level of admixtures. Even though, Kakkayam (protected area) and Pamba (extracted) populations are located at different elevations, both possess similar genetic structure with slight admixtures which might be due to human-mediated transfer of propagules (Fig. 1). Similarly, Peechi, Vazhachal, Malayatoor populations with similar genetic structure may be treated as a single population for better conservation and management of the resources. Presently, Vazhachal and Malayatoor are found as reed brakes, due to continuous reed extraction and human encroachment. The purpose of conservation is to ensure continued survival of populations as well as maintain their evolutionary potential through preserving genetic diversity (Desai *et al.*, 2013). The structuring pattern of *O. travancorica* constitutes an example of an endemic species originated initially from small parental populations. Higher level of genetic diversity at population level along with more number of ramets warrants conserving the viable reed bamboo stock of the Western Ghats. Overexploitation of *O. travancorica* is mainly due to unscientific management

practices followed during extraction and various anthropogenic activities in their distribution area. Large scale destruction of natural habitats has also led to irreversible loss of reed bamboo genetic diversity (Ravikanth *et al.*, 2008) which was formerly a continuous patch. Population structuring revealed the presence of four ancestral areas among the studied populations irrespective of the protective status. Thus, the present study recommends effective conservation and proper scientific extraction practices to be followed in the reed inhabited areas of the Western Ghats so that further enrichment of genetic stock can be accomplished in near future.

Acknowledgments

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