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ORIGINAL ARTICLE

Population genetic analysis illustrated a high gene diversity and genetic heterogeneity in *Himalayacalamus falconeri*: a socio-economically important Indian temperate woody bamboo taxon

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Abstract

Himalayacalamus falconeri is a socio-economically important temperate woody bamboo of north-western Himalayas, which has been investigated for genetic diversity and population genetic structure across distribution range in western Himalayas using genomic STMS markers. The calculated diversity measures have indicated a high gene diversity in *H. falconeri* at population level (Ho = 0.637; He = 0.714; Ar = 5.05). Despite the larger proportion of genetic variation (88%) confined within the populations, a moderate level of genetic differentiation ($F_{ST} = 0.121$) was detected with relatively lower gene flow (Nm = 1.891). Furthermore, clustering and STRUCTURE analysis displayed high genetic heterogeneity in a metapopulation, where populations in both the spatially disconnected regions of the Uttarakhand state, Garhwal and Kumaon, were clustered in different groups. Whereas, nested sub clustering and between-population genetic admixing were not correlated to their physical proximity. Also, the Mantel test supports the isolation by distance model showing a significant correlation between genetic and horizontal geographic distances. For conservation implications, diverse hotspots with high allelic richness were also identified in both the geographical regions of Uttarakhand state. To the best of our knowledge, it is a pioneer study presenting in depth knowledge of metapopulation in any Indian temperate bamboo, which will be of paramount importance to the researchers, foresters, and policymakers for guiding future conservation decisions of *H. falconeri* in the Indian Himalayan Regions (IHRs).

Keywords *Himalayacalamus falconeri* · Genetic differentiation · Genetic structure · Ringal · SSRs · Western Himalayas

Abbreviations

AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
AMSL	Above mean sea level
Ar	Allelic richness
BGRs	Bamboo genetic resources
F_{IS}	Inbreeding coefficient
F_{ST}	Fixation index
He	Expected heterozygosity
Ho	Observed heterozygosity
IDW	Inverse distance weighted
IHRs	Indian Himalayan regions
ISSR	Inter simple sequence repeat
MCMC	Markov chain monte carlo
Na	Number of different alleles
Ne	Effective number of alleles
NJ	Neighbour-joining
Nm	Number of migrants per generation
NW	North West
PAr	Private allelic richness
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PIC	Polymorphism information content
Pr(K)	Posterior probability of K
RAPD	Random amplified polymorphic DNA
SNPs	Single nucleotide polymorphisms

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SSRs Simple sequence repeats
STMS Sequence-tagged micro satellite

Introduction

Bamboos are imperative non-wood forest produce occurring in the forest as well as non-forest areas, and play a significant role in improving livelihoods of people globally (Hogarth and Belcher 2013; Kithan 2014; Khanal 2015). As described by Kelchner and Bamboo Phylogeny Group (2013), Bamboo Genetic Resources (BGRs) in India falls under two lineages, paleotropical (tribe Bambuseae) and temperate (tribe Arundinarieae) woody bamboos. Former occupied tropical and subtropical zone of the country from alluvial plains to hills up to the altitude of 1500 m AMSL. Whereas, later are dominating at high hills above 1500 m AMSL in the subtropical and temperate zone of Indian Himalayan Regions (IHRs). Among the temperate woody bamboos, four shrubby taxa (commonly called "*ringal*"), namely *Drepanostachyum falcatum*, *Himalayacalamus falconeri*, *Thamnocalamus spathiflorus*, and *Yushania anceps*, are naturally occurring in the NW Himalayas at the altitudinal range 1800 to 3600 m AMSL. *Ringal* forms a dense thicket or moderately dense undergrowth in the evergreen forest of the Himalayas, ubiquitously grown on steep slopes of riverbanks, and aids in soil stabilization with their fibrous root system (Kumari and Tewari 2009; Banik 2016). They serve as an important source of fodder in time of scarcity during winter and provide food for wild animals, such as red panda (*Ailurus fulgens*) and bears (Yonzon 1991).

Among the four hill bamboos of the NW Himalayas, culms of *Himalayacalamus falconeri* are most preferred by artisans for craft and weaving purposes due to their flexibility, strength, and smoothness. Beside the IHRs, it has also been reported to occur in the temperate zone of the Nepal, western Bhutan, and southwest China or Tibet (Banik 2016). Moreover, it has been introduced in New Zealand which was naturalized later in 1935 (Sykes 1996). In the western Himalayas, it is commonly known as "*Dev* (meaning God) *ringal*" due to its traditional utilization for making temple-related basketry or decorative articles. Socio-economically, *ringal* plays a crucial role in livelihood generation and offers employment opportunities for the people inhabiting at high hills of the Himalayas (Kumar 2009). About 451 villages have been identified in the Uttarakhand where artisans are involved in bamboo or *ringal*-based occupation. Dejectedly, the communities involved in this profession do not own the resources and about 93% of the raw material is directly harvested from the natural forest (Sundriyal and Sundriyal 2011). As a result, local populations nearby human settlements are severely deteriorating and people are struggling hard to collect the raw material illegally from protected forest areas. Awfully, selective and indiscriminate extraction from the natural forest may put the species in a miserable situation in long term. To ensure the sustainable utilization of this valuable genetic resource, baseline information and genetic characterization need to be derived for its natural populations, which is inevitable to frame a sound conservation and management plan.

Genetic variability among individuals ensures the long-term survival of metapopulation, bestows the evolutionary and adaptive potential against changing environment, and serves as a resource for future breeding programmes (Booy et al. 2000; Porth and El-Kassaby 2014; Godhe and Rynearson 2017; Nonić and Šijačić-Nikolić 2021). Explicitly, the spatial genetic structure is defined as the non-random distribution of genetic variation among individuals within populations which may or may not be shaped as per the geographical proximity of individuals, and some species may display a cryptic spatial structure with noticeable genetic heterogeneity due to unidentified migrants (Manel et al. 2003). In addition, the genetic structure of a metapopulation is also influenced by the extent and amount of gene flow across the spatially disconnected populations, which is controlled by several evolutionary processes and life history traits of a species (Porth and El-Kassaby 2014). Hence, estimation of gene diversity and understanding of the genetic structure is immensely important to elucidate the threat status of the species, vulnerability to extinction, and guiding precise conservation decisions (Andelman and Willig 2002; DeSalle and Amato 2004).

As phenotypic evaluation in natural populations is more laborious and error-prone, molecular markers are the most favoured tools for performing genetic analysis in wild plant populations (Nadeem et al. 2018). However, due to limited sequence information, earlier genetic studies in most bamboo taxa employed markers based on random primers, such as RAPD, AFLP, and ISSR (Yang et al. 2012; Ma et al. 2013; Nag et al. 2013; Nilkanta et al. 2017). Comparatively, sequence-based markers, such as STMS or SSR markers and SNPs, are more informative, robust, reproducible, and codominant (Nadeem et al. 2018). Unlike random primer-based markers, STMS is derived from the unique flanking sequence of repeat loci which share a high level of homology among taxonomically related taxa (Saha et al. 2004; Sharma et al. 2008). Hence, cross-transferability is a fast and cost-effective approach for identifying STMS markers in related taxa (Barbara et al. 2007), which has been successfully utilized in several bamboo species also (Barkley et al. 2005; Sharma et al. 2009; Mason 2015; Meena et al. 2019). Moreover, microsatellite markers have been developed in several bamboo taxa using different methodologies (Abreu et al. 2011; Peng et al. 2013; Bhandawat et al. 2015; Cai et al. 2019; Meena et al. 2021), which could be further utilized in other bamboo species through cross-transferability.

The present study aimed to quantify and map the genetic diversity, and determine the spatial genetic structure of the natural populations of *H. falconeri* in the western Himalayas using SSR markers.

Materials and methods

Study area, population sampling, DNA extraction and marker genotyping

The study was carried out in Uttarakhand (India) state which is represented by biographic zone 2B western Himalaya and 7B Shivalik consisting of Kumaon and Garhwal regions (Rodgers and Panwar 1988). Geographically, a major portion of the state is constituted by the Himalayan Mountain, which is one of the youngest mountain systems of the world (~40 million years in age compared to peninsular mountains of ~1500–2500 million years old); hence, ecologically very fragile (Uttarakhand Biodiversity Board, Dehradun; https://sbb.uk.gov.in/). The flora of the state ranges from tropical deciduous to alpine vegetation, and the key dominant tree species occurring in the temperate zones of the state are *Quercus leucotrichophora*, *Q. floribunda*, *Q. semecarpifolia*, *Rhododendron arboreum*, *Myrica esculenta*, *Lyonia ovalifolia*, *Ilex dipyrena*, *Pinus roxburghii*, *P. wallichiana*, *Cedrus deodara*, *Abies pindrow*, *Picea smithiana*, *Taxus wallichiana, Betula utilis*, etc. Ringal is a group of understory shrubby bamboos that are naturally interspersed with above trees species and connect the different forest types.

Leaf samples were collected from 219 individuals of *H. falconeri* from nine locations in the Garhwal and Kumaon Himalayas of Uttarakhand (Table 1), and desiccated with silica gel. In accordance with most studies on population genetic analysis, we attempted to maintain the sample size of about 30 individuals per population; however, it could not be accomplished for all due to reduced and deteriorated conditions. Within a population, sampling was conducted in compliance with the assumption followed in the method of McClure (1966), where each population is presumed to maintain the random assortment of genetic material considering individual clump as a potential genet and culms within it as ramets. To minimize the sampling bias, the minimum distance between two sampled individuals per location was kept at 100 to 300 m. Genomic DNA was extracted from silica dried leaf tissues using the protocol given by Doyle and Doyle (1987) with slight modification as per Krizman et al. (2006).

In a recent study, STMS markers were developed in another Himalayan temperate bamboos *Drepanostachyum falcatum* through genome skimming approach, and some of these have also been verified for their transferability in *H. falconeri* (Meena et al. 2021). These were re-examined for the level of polymorphism through PCR amplification in 20 random individuals of *H. falconeri*. The PCR products of each primer pair were subjected to gel electrophoresis with 4% high resolution agarose gel. After visualizing banding pattern, ten highly polymorphic STMS loci were finally selected for further genotyping (Table 2). The PCR reaction mixture was prepared by mixing of 2 µL genomic DNA (25 ng) with 1.5 µL enzyme buffer (10×), 1.8 µL $MgCl_2$ (25 mM), 1.2 µL dNTPs (2.5 mM), 0.15 µL primers (20 µM) and 0.2 µL *Taq* DNA Polymerase enzyme (3 U μL^{-1}) (Genei, India). Finally, a total reaction volume of 15 µL was made up of nuclease-free sterile water. The PCR reactions were run in the thermal cycler machine (Eppendorf Mastercycler Nexus) with an initial denaturing step (95 °C for 5 min) followed by 35 cycles of 94 °C for 1 min, 55–62 °C for 1 min and 72 °C for 1 min, and then final elongation at 72 °C for 10 min. The PCR products were loaded into automated capillary electrophoresis system LabChip GX Touch 24 (PerkinElmer, USA), and fragment analysis was carried out with the software LabChip GX reviewer ver. 5.8 (PerkinElmer, USA).

Table 1 Geo-spatial detail of sampled populations of *H. falconeri*

Sl. no.	Pop code	Location	Districts	Number of samples	Latitude (N)	Longitude (E)	Altitude (m)
1	HF01	Munsyari	Pithoragarh	30	30° 04′ 02.4″	80° 13′ 51.9″	2349
2	HF02	Ghes	Chamoli	30	30° 7′ 14.02″	79° 43′ 15.0″	2314
3	HF03	Ranachatti	Uttarkashi	24	30° 55′ 35.1″	78° 22′ 52.9″	1950
4	HF04	Pinswad	Tehri Garhwal	10	30° 39′ 37.2″	78° 39′ 46.1″	2321
5	HF05	Sunderdhunga	Bageshwar	30	30° 10′ 1.3″	79° 55′ 27.0″	2602
6	HF06	Mornaula	Almora	7	29° 26′ 26.6″	79° 45′ 51.1″	2145
7	HF07	Chopta	Chamoli	30	30° 27′ 44.1″	79° 13′ 59.01″	2538
8	HF08	Triyuginarayan	Rudraprayag	28	30° 37′ 3.74″	78° 58′ 53.59″	2032
9	HF09	Darma Valley	Pithoragarh	30	30° 05′ 33.0″	80° 37′ 14.60″	2222

Table 2 Characteristics of 10 polymorphic SSR markers used for genotyping the sampled populations of *H. falconeri*

Sl. no.	Locus name	Primer sequence (5'-3')	Repeat motif	Ta (°C)	Expected product size (bp)	Observed product size (bp)	No. of alleles per loci	Polymorphism information content (PIC)
1	*Df*Stm1292	F: TGCCCGATCCTCCAAAACTC R: TAGCCACTCTCCTCAGACGG	(AG)6	62	190	169–265	49	0.894
2	*Df*Stm732	F: AGGTAGCTAAGGCGCTAAGC R:GCCGACCGCGATGTAATAGT	(GGTGGA)6	62	191	151–376	15	0.857
3	*Df*Stm1461	F: CAGTACACTCGCCTCATCGT R: TTGCTCTAGGCTCTAGCGTG	(CT)15	62	206	171–341	52	0.945
4	*Df*Stm1445	F: TAGTACTGTTACCCCGCTGC R: AGTAGGTCATCATGCATGCTTG	(TTCC)6	62	189	175–300	14	0.798
5	*Df*Stm1712	F: CTCGTTCCTCTCGCGTTGTA R: TGTCTCCTCTGGTGTAGGCA	(TC)6	62	191	161–273	43	0.922
6	*Df*Stm307	F: TCCTCTTACGGAGTTCATCCCT R: GCCACTTCATCTCTTTTGCCG	(ATAA)5	56	197	196–218	7	0.418
7	*Df*Stm1651	F: ATCCTTGTTGGCCCCCTATG R: GCTTGTACTCCTCGGTGACC	(GTG)5	57.2	212	145–250	16	0.650
8	*Df*Stm1800	F: CCGGTAACTGCATGCATTGT R: CGAGCTATAGTGCCTGCTCC	(AG)9	55	209	180–330	31	0.842
9	*Df*Stm586	F: AATTGGCTTGGTGGGGAGAG R: TTCTCCTGCTCCGCTCATTG	(TC)…(TC)6	62	210	174–278	39	0.934
10	*Df*Stm1303	F: TCATGCTTCCTTGCTCCAGC R: GCGTGACTTCGACCAGAGAA	(CT)8	58.3	179	163–293	33	0.863

Marker data analysis for deciphering diversity measures and spatial genetic structure

The non-integer allelic data were transformed into the integers by applying the power function, and the allele sizes were binned as per the periodicities of repeat motifs using software TANDEM ver 1.07 (Matschiner and Salzburger 2009). The marker data were analyzed to calculate the various diversity measures, such as polymorphic information content (PIC), number of different alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho) and expected heterozygosity (He), etc., using software PowerMarker ver 3.25 (Liu and Muse 2005) and GenAlex ver. 6.5 (Peakall and Smouse 2012). Whereas, the software Arlequin ver. 3.1 (Excoffier et al. 2005) was used to perform AMOVA and estimation of Wright's fixation index (F_{ST}). In order to quantify the distribution of variance among different levels, the AMOVA was performed with and without assuming hierarchical structuring. The hierarchical structuring was carried out by considering three levels, i.e., among regions (Garhwal and Kumaon), among populations within groups, and within populations. Further, the measures of allelic diversity, such as allelic richness (Ar) and private allelic richness (PAr), were calculated using software HP-Rare

ver. 1.0, which gives an unbiased estimate of allelic richness by compensating sampling disparity using statistical technique of rarefaction (Kalinowski 2005), and spatially overlaid over distribution map after interpolating with IDW algorithm used in ArcGIS (Shepard 1968; Hengl 2009; Chiocchini et al. 2016).

The genetic relationship among sampled populations was studied through the neighbour-joining (NJ) clustering and the principal coordinate analysis (PCoA) using software POPTREE2 (Takezaki et al. 2009) and GenALEx, respectively. In order to test the isolation by distance model, the Mantel test (Mantel 1967) was performed between genetic and geographical distances. Herein, genetic distances were correlated with horizontal as well as vertical altitudinal distances between the sampled populations. To unravel the population genetic structure, the Bayesian model-based clustering method was implemented in the software STRUCTURE ver. 2.2 (Pritchard et al. 2000), where the posterior probability of K [Pr(K)] was calculated using an ancestry model with admixture under the assumption of correlated allele frequencies. The model was replicated ten times for each K value (2 to 9) with 3,00,000 MCMC sampling runs after a burn-in period of 3,00,000 iterations. Finally, an optimal number of subpopulations were determined using the web-based program Structure Harvester 0.6.92 (Earl and vonHoldt 2012).

Results

Marker polymorphism and gene diversity in sampled populations

Polymorphism of successfully transferred STMS markers of *D. falcatum* was reconfirmed in *H. falconeri* through PCR amplification in 20 random samples. Based on the polymorphism and distinctness of the banding pattern on agarose gel (Supplementary Fig. S1), ten marker loci were selected for further genotyping. A total of 299 alleles were generated by genotyping with ten STMS markers, where highest 52 alleles were displayed by the marker *Df*Stm1461 while lowest seven alleles were obtained with *Df*Stm307. By analysing allelic polymorphism across the populations, a mean of different and effective number of alleles per population were recorded as 9.9 and 5.2, respectively. Accordingly, all marker loci demonstrated a high level of polymorphism with PIC value ranging from 0.418 for *Df*Stm307 to 0.945 for *Df*Stm1461 (Table 2).

Calculated diversity indices revealed a high level of gene diversity in the sampled populations of *H. falconeri* (Table 3). For instance, observed heterozygosity (Ho) was ranged from 0.540 (HF04_Pinswad) to 0.727 (HF01_Munsyari) with a mean of 0.637, and expected heterozygosity (He) was ranged from 0.591 (HF06_Mornaula) to 0.810 (HF08_Triyuginarayan) with a mean of 0.714. Also, the population HF08 from Triyuginarayan exhibited highest allelic richness (Ar = 5.77), whereas it was recorded as lowest (Ar = 4.43) for the population HF02 from Chopta. Further, spatial overlaying of allelic diversity has enabled to distinguish the populations or regions of conservation importance, where the values of diversity measures are depicted in hypsometric scale (Fig. 1a, b). Decisively, the populations or the regions capturing high allelic richness were designated as diversity hotspots, and one diversity

Table 3 The calculated measures of gene diversity for sampled population of *H. falconeri*

Pop code	Location detail	N	Na	Ne	Ho	He	Ar	PAr
HF01	Munsyari	30	12.2	5.9	0.727	0.748	5.10	1.19
HF02	Ghes	30	12.1	6.2	0.700	0.785	5.32	0.87
HF03	Ranachatti	24	10.0	4.9	0.583	0.695	4.90	0.87
HF04	Pinswad	10	6.6	3.9	0.540	0.700	4.79	0.76
HF05	Sunderdhunga	30	11.1	5.9	0.657	0.729	5.10	1.34
HF06	Mornaula	7	5.5	4.2	0.571	0.591	5.45	1.30
HF07	Chopta	30	9.1	4.1	0.610	0.681	4.43	0.63
HF08	Triyuginarayan	28	12.7	7.4	0.689	0.810	5.77	1.05
HF09	Darma Valley	30	9.7	4.5	0.653	0.690	4.59	0.97
	Overall mean	24	9.9	5.2	0.637	0.714	5.05	1.00

N, Number of sampled individuals; Na, Number of different alleles; Ne, Effective number of alleles; Ho, Observed heterozygosity; He, Expected heterozygosity; Ar, Allelic richness; PAr, Private allelic richness

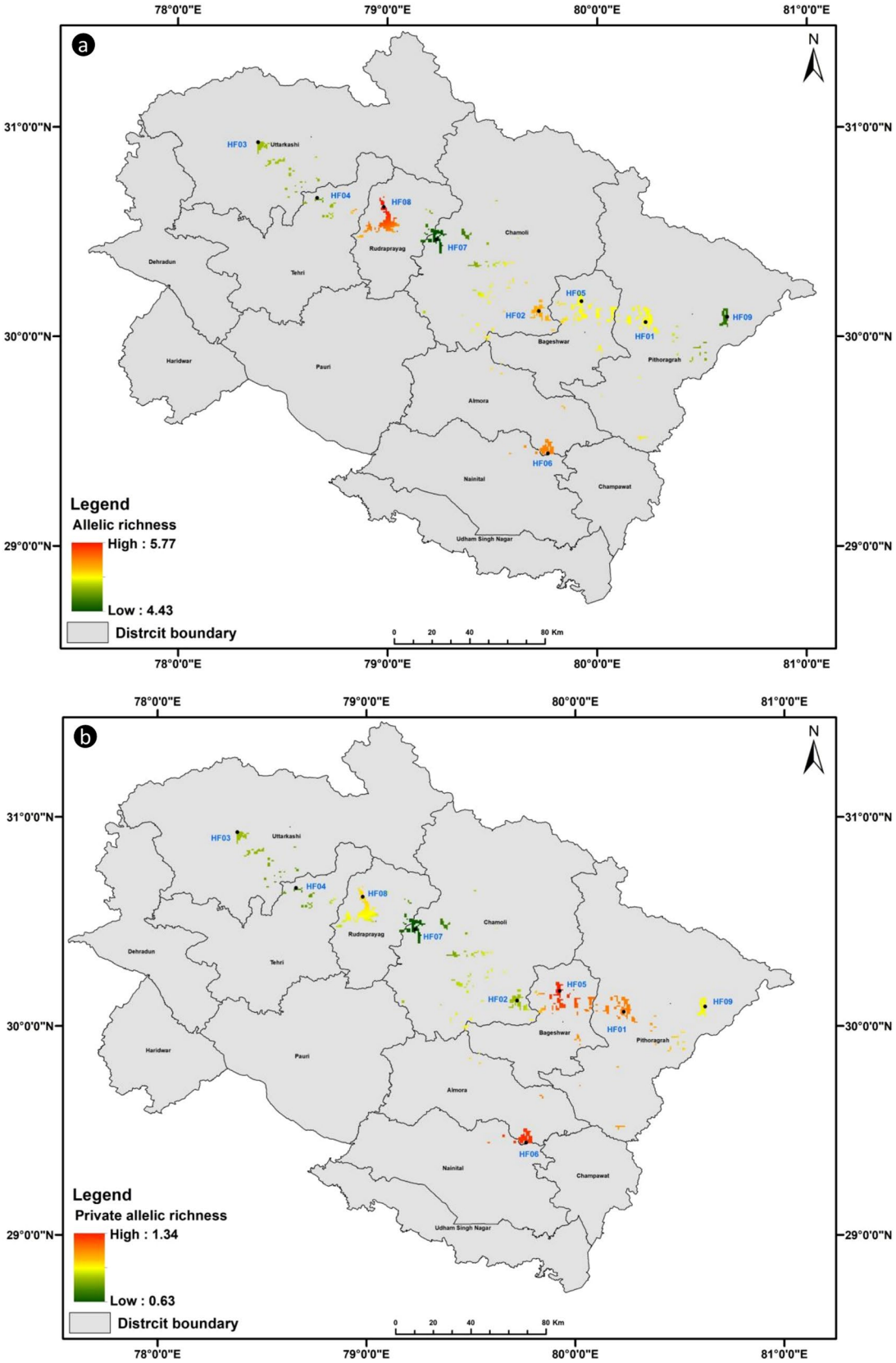

Fig. 1 Overlaying of allelic richness (**a**) and private allelic richness (**b**) over distributing map of *H. falconeri* in the Uttarakhand Himalayas. The area shaded with warmer and cooler colours in map reflects high and low diversity indices, respectively

hotspot has been recognized in both the geographical regions of Uttarakhand Himalayas, i.e., HF08 (Triyuginarayan) in Garhwal and HF06 (Mornaula) in Kumaon, for their in-situ conservation. Importantly, most populations distributed in Kumaon region were identified to possess significant number of private alleles, and therefore, entire region can be considered important for conservation.

Genetic relationship, divergence and structuring in sampled populations

The results of AMOVA without hierarchical structure have demonstrated that the major proportion of genetic variance (87.90%) existed within the populations, and only 12.10% was observed among populations (Table 4a). Accordingly, a moderate level of genetic differentiation ($F_{ST} = 0.121$) was recorded with relatively lower gene flow (Nm = 1.891). However, AMOVA with hierarchical structuring showed that the 87% of the total variance was contained by the individuals within populations, 11% was existed among populations and only 3% was between the regions (Table 4b). Based on the pairwise F_{ST} and Nm values (Supplementary Table S1), the highest genetic distance was recorded between the population HF06 (from Mornaula) and HF09 (from Darma valley), whereas it was observed as lowest between the population HF01 (from Munsiyari) and HF02 (from Ghes). Plausibly, an inverse trend of gene flow was displayed among the populations, i.e., the gene flow was highest between populations which are least genetically distant, and vice-versa.

The genetic divergence revealed by AMOVA and F-statistics was further validated with the cluster and structure analysis. Unrooted NJ dendrogram showed two major groups, where populations of both the regions, i.e., Garhwal and Kumaon, were clustered in distinct groups in accordance with their geographical distribution (Fig. 2). Similar pattern was also displayed by the spatial clustering in the PCoA plot (Fig. 3), where all the three coordinates cumulatively accounted for a substantial level of genetic variance (69.35%). Individually, first, second, and third coordinates, were accounted for 25.4, 22.57, and 21.36% of the genetic variance, respectively. Conclusively, overall genetic divergence was well supported by the cluster analysis but the structuring among geographical regions was not evident in AMOVA with hierarchical structuring. The genetic divergence among spatially separated populations were further supported by the Mantel test, where the correlation of genetic distance was significant with horizontal geographical distance (R^2=0.332; *P* value = 0.001) (Fig. 4) and non-significant with vertical altitudinal distances (R^2 = 0.025; *P* value = 0.213) (Supplementary Fig. S2).

Surprisingly, structure analysis has revealed five optimum numbers of subpopulations (Fig. 5a, b), indicating considerable amount of heterogeneity among the geographically disconnected populations (Fig. 2). The proportional membership coefficient of each population was calculated for five inferred clusters, and pattern of genotypic admixing is displayed in the form of a bar plot (Fig. 5c). As per the population Q-matrix, seven sampled populations were clearly defined by one of the five inferred clusters with a proportional membership coefficient of more than 0.70. Whereas, two populations of the Garhwal region, namely HF04 (from Pinswad) and HF08 (from Triyuginarayan), exhibited admixed ancestry from other inferred clusters (Supplementary Table S2). As evident in supplementary table S1, the high genotypic admixing in both the populations would have been resulted due to significantly high gene flow with other populations.

Discussion

The fitness of individual population and spatial distribution of the genetic variation are mainly controlled by the gene flow, i.e., exchange of genetic material between the

Table 4 Analysis of molecular variance (AMOVA) for five populations of *H. falconeri*

Source of variation	Degree of freedom	Sum of Square	Estimated variance	Percent variation	Genetic differentiation
(a) Partitioning of the variance assuming no hierarchical structure					
Among populations	8	225.571	0.511	12.10	$F_{ST} = 0.121$
Within populations	429	1593.705	3.715	87.90	
Total	437	1819.276	4.226	100	
(b) Partitioning of the variance assuming hierarchical structure					
Among regions	1	51.800	0.112	3%	$F_{ST} = 0.131$
Among populations	7	174.209	0.450	11%	
Within populations	429	1594.938	3.718	87%	
Total	437	1820.947	4.280	100%	

The variance estimated with 1023 permutations between the individuals within populations were statistically significant ($P < 0.001$)

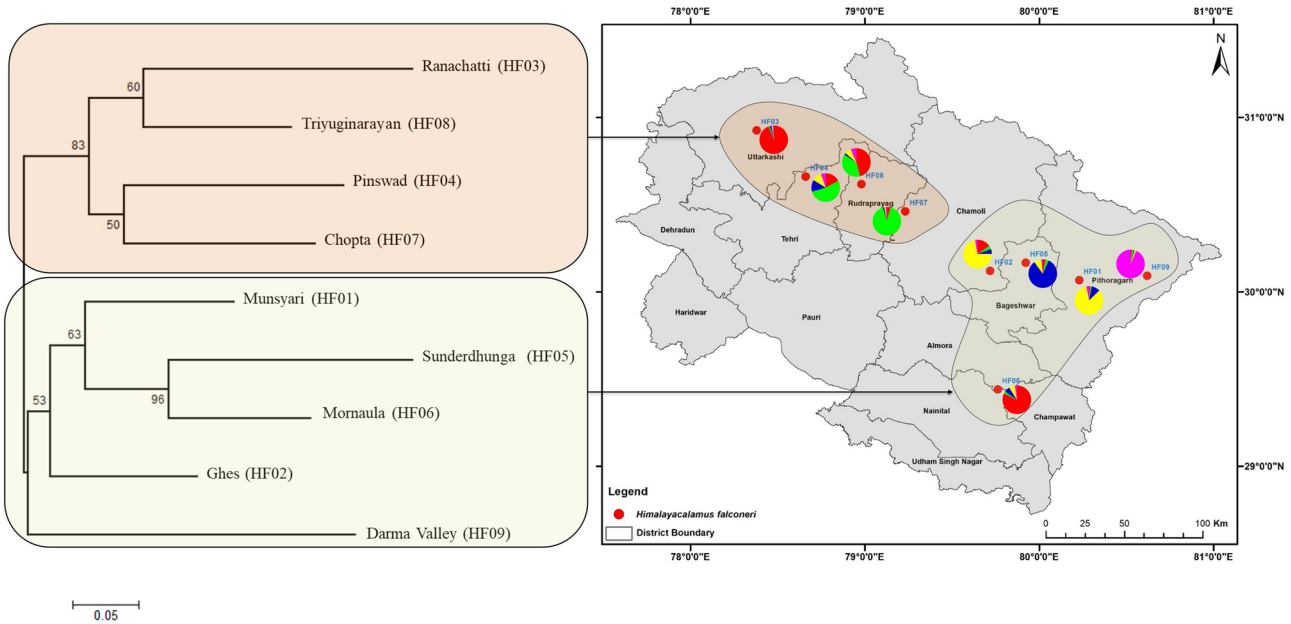

Fig. 2 Unrooted Neighbour Joining (NJ) tree showing genetic and spatial clustering between sampled populations of *H. falconeri.* Pie chart overlayed over distribution map shows pattern of genetic admixture among five inferred genetic clusters

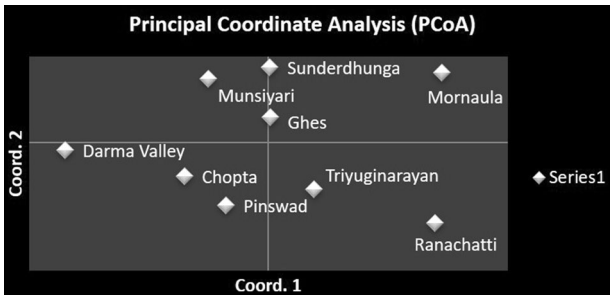

Fig. 3 Spatial genetic clustering of studied populations through principal coordinate analysis

populations (Porth and El-Kassaby 2014; Xie et al. 2019; Bontrager and Angert 2019; Luo et al. 2021; Morente-López et al. 2021), which itself controlled by multitude of factors like topography, environmental, geographical, biological characteristics of species, etc. (Wu et al. 2015; Ghareha-ghaji et al. 2017; Bontrager and Angert 2018; De Kort et al. 2021;). Among biological attributes, breeding behaviour and life cycle characteristics are the major factors affecting genetic composition of the populations. In general, an open-pollinated species with widespread distribution tends to capture more genetic diversity than a self-pollinated and narrowly distributed species (Hamrick et al. 1992; Hamrick and Godt 1996). For instance, a high level of genetic differentiation and heterogeneity were recorded in a metapopulation of *Ochlandra travancorica*, an endemic bamboo of Western Ghat, India (Nag et al. 2013). Being the open-pollinated breeding behaviour and wide distribution in the Himalayan ranges, high gene diversity with low genetic differentiation was expected in *H. falconeri* metapopulation. In congruence, this study has recorded high gene diversity (He = 0.714; Ar = 5.05) in the sampled population of *H. falconeri* from the western Himalayas. However, high genetic heterogeneity detected herein has indicated the noticeable hindrance in gene flow and substantial genetic changes adopted by the populations.

Though the overall gene diversity is high, populations HF06 from Mornaula in Kumaon and HF07 from Chopta in Garhwal region have exhibited relatively lesser gene diversity. Population HF06 is located in the reserve forest at Champawat forest division, which has demonstrated lesser gene diversity and high allelic richness. This disparity in both the diversity measures could have been aroused due to suboptimal sample size. Whereas, the population HF07 belongs to the Kedarnath wildlife sanctuary, one of most healthy and intact reserve forests of the state, showed relatively low allelic diversity. The reason for poor allelic richness in this region could be the excessive extraction of *ringal* culms, as this is one of the most popular sites among *ringal* artisans, where all four species are adequately found. However, the population of Triyuginarayan (HF08) showed the highest gene diversity. Further, diversity hotspots demarcated in the diversity maps could be prioritized for conservation and management. For instance, the populations with high allelic richness, namely HF08 (Triyuginarayan)

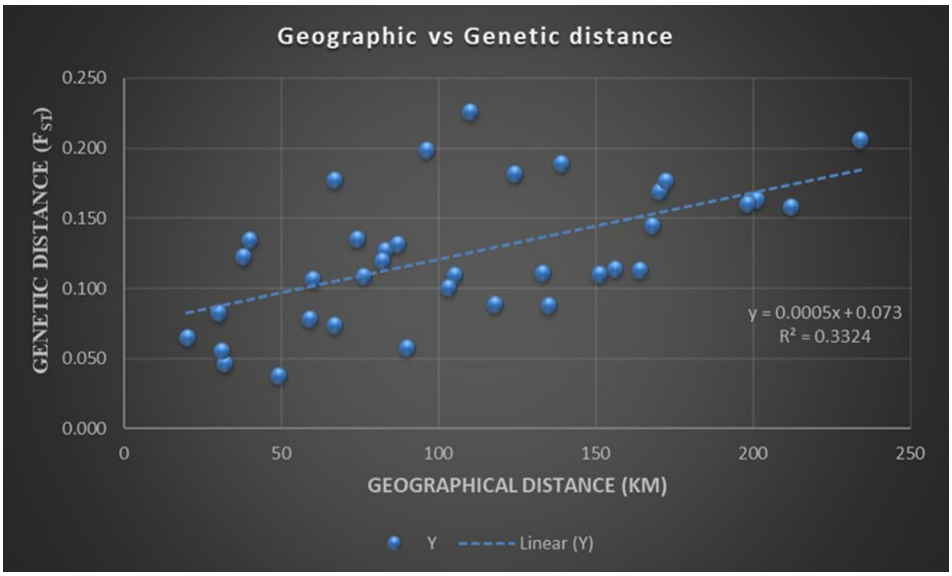

Fig. 4 Relationship between genetic and horizontal geographic distance for studied populations of *H. falconeri* through Mantle test

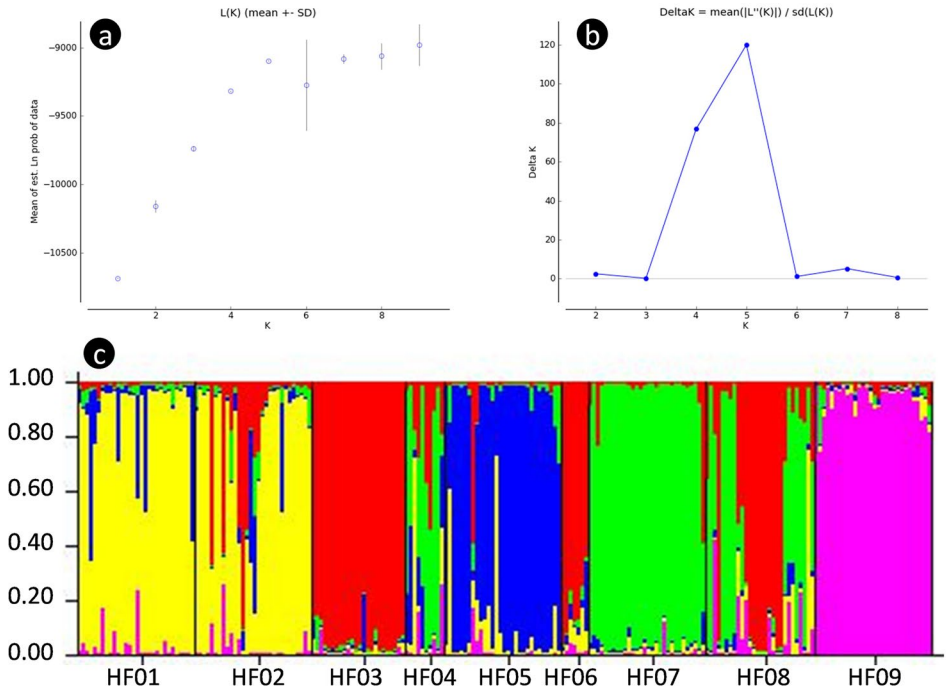

Fig. 5 Graphical representation of the estimated Ln probability of data (**a**) and ΔK (**b**) for each K value, Bar plot (**c**) illustrated the proportional membership coefficient for all the genotypes at K = 5, where each population is separated by a vertical line and individual samples are represented by coloured bars

and HF06 (Mornaula) are designated as diversity hot spots and the populations exhibiting lower allelic diversity HF07 (Chopta) and HF09 (Darma Valley) are considered as genetically fragile. Interestingly, most populations of the Kumaon region contained substantial number of private alleles, and therefore, entire region is important from the conservation point of view. Based on the current diversity statistics, the metapopulation of the studied species is observed to be genetically healthy with sufficient evolutionary or adaptive potential. The diversity measures observed in the present study were found consistent with the Sri Lankan temperate woody bamboo *Kuruna debilis* (He = 0.708; Ar = 4.58; F_{ST}=0.113), characterized with twelve microsatellite loci (Attigala et al. 2017). However, the population genetic studies in other bamboo taxa, viz., *Dendrocalamus membranaceus* (Yang et al. 2012), *O. travancorica* (Nag et al. 2013), *Melocanna baccifera* (Nilkanta et al. 2017), *P. edulis* (Jiang et al. 2017), *D. sinicus* (Yang et al. 2018), *D. hamiltonii* (Meena et al. 2019; Bhandawat et al. 2019), *Oxytenanthera abyssinica* (Oumer et al. 2020), etc., displayed relatively lower genetic diversity with high genetic differentiation. Recently, high genetic diversity and differentiation were also

detected in three native Mexican woody bamboo species of genus *Guadua* (Pérez-Alquicira et al. 2021).

Furthermore, unique life cycle characteristics of bamboo such as the long vegetative phase of 3 to 150 years and strong capability of asexual reproduction (Zheng et al. 2020), make them different from other forestry species where general principles of population genetics may not be obeyed. When the conditions of Hardy-Weinberg Equilibrium (HWE) do not fulfil, populations may undergo genetic differentiation over the period. In the case of bamboo in general, a key HWE condition, i.e., random mating, not always be ensured in the metapopulation due to their indistinct breeding behaviour. Though the floral biology and breeding behaviour is not studied in *H. falconeri*, most woody bamboo taxa are reproduced through open pollination (Ruiz-Sanchez et al. 2017; Chen et al. 2017), and flowered either, sporadically or gregariously or both, at different time intervals (Banik 2016; Zheng et al. 2020). The *ringal* bamboo species flowers gregariously with a periodicity of 28 to 35 years (Troup 1921; Campbell 1988). However, few flowering culms may be spotted in a population almost every year. Both sporadic and gregarious flowering has also been reported in *H. falconeri*, and a recent event of gregarious flowering in this taxon was recorded in 2002 at some cohorts in Uttarakhand Himalayas (Naithani et al. 2003). The longer and unsynchronized flowering cycle within and across the population act as a temporal barrier, and only the simultaneously flowered populations get a chance of intermating. In an open-pollinated plant taxon, the flowering synchrony increases reproductive success while asynchronous or sporadic flowering negatively affects the population's fitness (Rodríguez-Pérez 2016; Bogdziewicz et al. 2020; Pérez-Alquicira et al. 2021). As a result, a high level of genetic heterogeneity was observed in structure analysis, where the entire variability was divided into five subpopulations.

Remarkably, the genetic clustering derived through NJ dendrogram and PCoA plot revealed two major clusters, where the overall clustering was appeared in accordance to their spatial distribution, i.e., the populations of Garhwal and Kumaon regions of Uttarakhand Himalayas were categorised into different groups (Fig. 2). Further, Mantel test also supports the isolation by distance model, signifying the imperative role of physical distance in distribution of the genetic diversity across the range. As per the surveyed area in this study, species distribution has been recorded from 29° 26′ to 30° 55′ in north and 78° 22′ to 80° 37′ in east, and altitudinally from 1950 m at Ranachatti to 2600 m AMSL at Sunderdhunga. Thus, both horizontal and vertical distances were analysed against the genetic distances, where only horizontal distance displayed significant relationship. It suggests that the geneflow is adequate across the altitudinal gradient but limited on longitudinal range due to various topographical, environmental or biological constraints. A similar clustering pattern and correlation were also observed in *D. hamiltonii*, where the populations of different regions were clustered in different groups (Meena et al. 2019). However, the nested sub clustering, i.e., clustering among the populations of smaller areas was not appeared to be correlated with their physical proximity, possibly due to asynchronous flowering among populations.

Conclusions and conservation implications

The present study has demonstrated a high gene diversity in the natural populations of *H. falconeri* of Uttarakhand Himalayas with a moderate level of genetic differentiation, indicating substantial evolutionary and adaptive potential of the species. However, the populations with suboptimal size and diversity like DH06 need special conservation attention. If the natural habitat continues to deteriorate, the alleles of such populations could be rescued by infusing into a large healthy population with a wide genetic base, such as protected areas. As evident by the diversity map of private alleles, the populations located in the Kumaon region harboured most private alleles, and require suitable conservation measures. Viewing the pattern of genetic clustering and structure analysis, it is appeared that the ecological or geographical factors played a crucial role in the structuring of metapopulation at a large distribution range. Whereas, the genetic make-up of closely located subpopulations in smaller geographical area, is appears to be controlled by synchronized flowering episodes. Further understanding of genetic structure demands an up-to-date record of past flowering data, and therefore, the recording of flowering episodes is very crucial in bamboo. Also, the detailed analysis of phenology, mating system, and contemporary gene flow, is important to understand the reason behind high genetic heterogeneity.

Considering a high level of genetic heterogeneity in *H. falconeri* metapopulation, conservation of any one population alone would not serve the purpose, and for *ex-situ* conservation, germplasm must be pooled from all the major clusters depicted in structure analysis. In addition, populations, namely HF04 (Pinswad) and DH08 (Triyuginarayan) displaying significant genetic admixture may be treated as natural gene banks and recommended for in-situ conservation. The knowledge base generated here will be of paramount importance to the researchers, foresters, and policymakers for guiding future conservation and management plans of *H. falconeri* in IHRs.

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Author contributions RKM, MSB, and RS involved in study conceptualization, execution, sample collection, data analysis, interpretation and manuscript writing; NN conducted the laboratory work; RS contributed in generation of diversity maps.

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Data availability All the data required to understand the manuscript are provided in original manuscript and supplementary material.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests todisclose.

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