Genetic diversity, population structure, and traditional culture of *Camellia reticulata*

Tong Xin¹ * | Weijuan Huang¹**, ID | Jan De Riek² | Shuang Zhang¹ | Selena Ahmed³ | Johan Van Huylenbroeck² | Chunlin Long¹,4

**1**College of Life and Environmental Sciences, Minzu University of China, Beijing, China
**2**Plant Sciences Unit, Institute for Agricultural and Fisheries Research, Melle, Belgium
**3**Department of Health & Human Development, Montana State University, Bozeman, MT, USA
**4**Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China

**Correspondence**
Chunlin Long, College of Life and Environmental Sciences, Minzu University of China, Beijing, China.
Email: long.chunlin@muc.edu.cn

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**Abstract**
*Camellia reticulata* is an arbor tree that has been cultivated in southwestern China by various sociolinguistic groups for esthetic purposes as well as to derive an edible seed oil. This study examined the influence of management, socio-economic factors, and religion on the genetic diversity patterns of *Camellia reticulata* utilizing a combination of ethnobotanical and molecular genetic approaches. Semi-structured interviews and key informant interviews were carried out with local communities in China’s Yunnan Province. We collected plant material (*n* = 190 individuals) from five populations at study sites using single-dose AFLP markers in order to access the genetic diversity within and between populations. A total of 387 DNA fragments were produced by four AFLP primer sets. All DNA fragments were found to be polymorphic (100%). A relatively high level of genetic diversity was revealed in *C. reticulata* samples at both the species (*H* *
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sp = 0.3397, *I* *
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sp = 0.5236) and population (percentage of polymorphic loci = 85.63%, *H* *
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pop = 0.2937, *I* *
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pop = 0.4421) levels. Findings further revealed a relatively high degree of genetic diversity within *C. reticulata* populations (Analysis of Molecular Variance = 96.31%). The higher genetic diversity within populations than among populations of *C. reticulata* from different geographies is likely due to the cultural and social influences associated with its long cultivation history for esthetic and culinary purposes by diverse sociolinguistic groups. This study highlights the influence of human management, socio-economic factors, and other cultural variables on the genetic and morphological diversity of *C. reticulata* at a regional level. Findings emphasize the important role of traditional culture on the conservation and utilization of plant genetic diversity.

**KEYWORDS**
*Camellia reticulata*, ethnobotany, genetic diversity conservation, traditional culture

**1 | INTRODUCTION**

Biodiversity is fundamental for life on earth by supporting the health of ecosystems (Mace et al., 2014). However, rapid economic development, social and cultural shifts, habitat loss, overexploitation of resources, climate change, and pollution are posing serious threats to biodiversity (Cardinale et al., 2012) including eliminating the plant genetic resources that support life on earth (Garcia, Gabeza, Rahbek, & Araujo, 2014; Rands et al., 2010). Effective conservation...
of biodiversity is called for to support the maintenance of ecosystem processes that ultimately sustain human life (Khairi, 2016).

Traditional cultural practices and local ecological knowledge of smallholder farmers and indigenous communities that has accumulated over generations have been widely recognized to contribute to biodiversity conservation (Bohn, Diemont, Gibbs, Stehman, & Vega, 2014; Shen et al., 2012) and may potentially be tapped for addressing the rapid loss of biodiversity around the world. Previous studies have acknowledged the role of traditional knowledge and culture practices of smallholder farmers and indigenous communities for biodiversity conservation at the species, genetic, ecosystem, and landscape levels (Altieri, 2004). Many traditional management practices, customs, and beliefs have been reported to contribute to biodiversity protection including seed exchange systems (Labeyrie, Thomas, Muthamia, & Leclerc, 2011), marriage exchanges (Delêtre, McKey, & Hodkinson, 2011), religious rituals (Mazumdar & Mazumdar, 2012), and dietary traditions (Penafiel, Lachat, Espinel, Van Damme, & Kolsteren, 2011). These practices, customs, and beliefs have been linked to preserving crop landraces (Jackson, Pascual, & Hodkin, 2007), old trees (Salick et al., 2007), and economic plants (Liu et al., 2014) including those with esthetic, food, and medicinal values (Begum et al., 2014).

Camellia reticulata Lindl. (Theaceae), an evergreen arbor (Liu & Gu, 2011) known as “Yunnan shan-cha” in Chinese, has a long history of being cultivated by various sociolinguistic groups of China’s Yunnan Province including the Yi, Bai, Naxi, Hui, Miao, and Lisu for its ornamental, horticultural, and cultural values as well as for the oil derived from its seed (Xin et al., 2015). The Bai ethnic group cultivates C. reticulata in their gardens as a symbol of gentility, and the Yi pay special respect to camellias while communities in Tengchong extract an edible oil from the seeds (Wang & Ruan, 2012). C. reticulata is naturally distributed in Yunnan, southwest Sichuan and Guizhou Provinces of China (Ming, 2000). Morphologically, C. reticulata has attractive large flowers (7–18 cm in width) with bright red or pink petals (Li, Hashimoto, Shimizu, & Sakata, 2007) that have a long blossoming season in winter.

Camellia reticulata is a perennial, outcrossing, and heterogenous double ploidy species (2n = 30) (Ming, 2000). The cultivation of C. reticulata dates back to the Sui and Tang dynasties (~600 AD). This species has been widely described in classical literature including poems and inscriptions (Wang et al., 2011). Camellias are widely cultivated in Buddhist temples and offered to the Buddha (Xin et al., 2015). The British botanist J. Lindley identified and named Camellia reticulata in the Botanical Register in 1827 and introduced this species to Europe (Ming, 2000). By the 1950s, C. reticulata was found in the gardens of western countries and continues to be desired as an ornamental including by the potted flower industry (Hattan et al., 2016).

Managers of C. reticulata have bred this resource with other Camellia species to produce colorful cultivars with different colors and blossom periods (Zhou et al., 2014). During the process of polyploidization and hybridization, other Camellia species have likely contributed to the genetic diversity of C. reticulata (Hong, 2010; Wang & Ruan, 2012; Yuan, Cornille, Giraud, Cheng, & Hu, 2014) and resulted in over 500 cultivars and hybrids (Chen, Wang, Xia, Xu, & Pei, 2005). However, with rapid environmental and socio-economic change as well as a trend toward monocultures and reduced genetic diversity (Barrett, Travis, & Dasgupta, 2011; Cardinale et al., 2012), it is becoming important to understand the current status of plant genetic diversity of C. reticulata (Wang, Chiang, Roux, Hao, & Ge, 2007). While numerous studies have reported on the importance of traditional management practices for biodiversity conservation, the complex interaction between cultural practices and genetic diversity of C. reticulata has not been studied (Hong, 2010).

With the development of biotechnology, molecular marker analysis has been found to effectively assess genetic diversity (Thomas, Vijayan, Joshi, Joseph, & Raj, 2006). AFLP analysis has been widely used to characterize natural populations and breeding gene pools (De Riek, De Cock, Smulders, & Nybom, 2013; Roldán-Ruiz, Dendalew, Van Bockstaele, Depicker, & De Loose, 2000) and is considered highly informative for studying genetic diversity and phylogenetic relationships by identifying variations with high resolution at the level of an individual’s DNA (Agarwal, Shrivastava, & Padh, 2008; Costa, Pereira, Garrido, Tavares-de-Sousa, & Espinosa, 2016).

In this study, we integrated ethnobotanical and molecular genetic approaches to examine the influence of cultural factors on the conservation of genetic diversity of C. reticulata. Semi-structured and key informant interviews were carried out with managers of C. reticulata in central and western parts of Yunnan Province of China. Samples from five distinct populations of C. reticulata were collected from Yunnan in order to analyze the genetic diversity and population structure using AFLP analysis. It is expected that the genetic diversity of C. reticulata has been influenced by the cultural practices of various sociolinguistic groups who have managed, cultivated, and conserved this tree resource.

2 | MATERIALS AND METHODS

2.1 | Ethnobotanical survey and plant collections

Research was carried out in the main distribution and production areas of C. reticulata. This includes locations in central and western parts of Yunnan Province in five prefectures including the following: Kunming, Chuxiong, Dali, Tengchong (in Baoshao City), and Lijiang (Figure 1). Ethnobotanical methods including semi-structured interviews and key informant interviews were conducted to understand the history, culture, use, local names, and number of cultivars of C. reticulata. Informants were randomly selected from local community members in different socio-linguistic groups (including the Yi, Bai, Dai, et al. who have their own unique language and culture) at the study sites who were willing to participate in surveys. A total of 120 informants participated in this study including 38 from Kunming, 20 from Chuxiong, 25 from Dali, 26 from Tengchong, and 11 from Lijiang. In addition, we examined relevant literature regarding beliefs, traditional knowledge, and customs on C. reticulata at the study sites.

A total of 190 individuals from five populations were sampled from the five study sites. Their local name and the morphological characteristics including flower color, flower type, and blooming period were recorded according to ethnobotanical survey as well as plant database research (see Appendix S1).
2.2 | AFLP analysis of genetic diversity

Approximately 30–40 individuals of C. reticulata were collected from each population. From each sampled plant, young, healthy leaves were collected, silica-dried, and stored at −20°C for laboratory analysis of genetic diversity. Genomic DNA was extracted from 15 to 30 mg dried-leaf samples following modified CTAB method (Doyle & Doyle, 1990). AFLP was performed according to Vos et al. (1995). Each diluted DNA sample was digested with EcoRI and MseI and ligated to specific adapters using T4 DNA ligase. EcoRI + A and MseI + C primers were used for preamplification (Table S1). PCR reactions were performed in a GeneAmp PCR system 9700 (ABI, USA). The preamplification program consisted of 25 cycles of 30 s at 94°C, 1 min at 56°C, and 1 min at 72°C. Evaluation of the restriction digest, adapter ligation, and the preamplification occurred on a 1.5% TAE buffered agarose gel, using λ. Pst as size reference (Tables S2 and S3). Selective amplification was performed with four EcoRI/MseI primer combinations with six selective bases (fluorescent labeling of the EcoRI primers): EcoRI-ACC/MseI-CTC (PC3), and EcoRI-ACC/MseI-CTC (PC4). The amplification program consisted of 2 min at 94°C, 30 s at 65°C, 2 min at 72°C, followed by 8 cycles of 1 s at 94°C, 30 s at 64°C, and 2 min at 72°C (the temperature decreased after each cycle with 1°C, from 64°C to 56°C) and finally 23 cycles of 1 s at 94°C, 30 s at 56°C and 2 min at 72°C (Table S4). AFLP fragments were separated on a 3130xl Genetic Analyzer (Applied Biosystems). GeneMapper v4.0 (Applied Biosystems) was used to record the signal peak height, the signal area, and the fragment size. The data were exported to Access, and a scoring table was generated as a starting point for the data analysis. The bands were scored as either present (1) or absent (0) across all loci (see Appendix S2).

2.3 | Data analysis

Genetic diversity parameters were generated using the program GenALEX v6.5 (Peakall & Smouse, 2012) and POPGENE32 v3.01 (Yeh, Yang, Boyle, Ye, & Xiyan, 2000) in order to provide information on the percentage of polymorphic loci (P), Nei’s Gene Diversity Index (H; Nei,
1973) and Shannon’s Information Index (I; Frank, Furst, Koschke, Witt, & Maukeschín, 2013).

Nei’s genetic distances between populations (Nei, 1978) (also generated with POPGEN32) were used to construct unweighted pair group method arithmetic average (UPGMA) dendrograms in NTSYSpc 2.11 after running 100 replicates (McKinnon, Mørri, Blackman, & Lior, 2004). Genetic diversity (H) was calculated at two levels: the average diversity within populations (H_within) and the total diversity (H_total). The proportion of diversity among populations was estimated using the following equation: (H_total - H_within) / H_total. An analysis of molecular variance (AMOVA) was performed using Arlequin (Schneider, Roessli, & Excoffier, 2000) and analyzed from both among populations and within populations. The UPGMA dendrogram of populations based on AFLP fingerprints was drawn based on pairwise similarities using software TFPGA (Miller, 1997).

To further elucidate relationships among all populations, a model-based cluster analysis was performed using the program STRUCTURE v2.3.1 (Raj, Stephens, & Pritchard, 2014). STRUCTURE was run 20 times and was carried out by setting the number of clusters (K) from 1 to 14. The optimum number of clusters (K) was processed and identified by STRUCTURE HARVESTER through comparing log probabilities of data for each value of K (Earl & Voholdt, 2012; Stephens & Pritchard, 2003). The output of structure analyses was visualized using the software CLUMPP v1.1.2 (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015) and DISTRUCT v1.1 (Rosenberg, 2016). Data were input in an Excel spreadsheet in order to carry out statistical analysis and to make figures depicting genetic diversity and cultivar diversity of C. reticulata.

3 | RESULTS

3.1 | Ethnobotanical survey Camellia reticulata

Results of ethnobotanical survey have been published by the author (Xin et al., 2015) that highlighted the impact of traditional culture on the conservation of C. reticulata. In this survey, we found that there are 206 ancient trees of C. reticulata in Kunming, with 28% of them well maintained in old Buddhist temples including the Panlong temple and Huating Temple. In Chuxiong, 26 of the 72 old C. reticulata trees are maintained in Buddhist temples. In Lijiang, there are two different cultivars of old C. reticulata trees that have existed for over 500 years and are entitled as “the king of camellia.” Furthermore, there is an old C. reticulata in Dali that has grown for about 400 years in the highest altitude of 2,750 m among all Camellia species.

3.2 | Genetic diversity of Camellia reticulata

A total of 387 AFLP unambiguous bands (100%) with a gene size ranging from 50 bp to 455 bp were obtained from the 190 plant samples collected for this study with the four EcoRI/MseI primer sets (Table 1). The observed effective number of alleles (N_e), Nei’s Gene Diversity (H), Shannon’s Information Index (I), total genetic diversity (H_total), genetic diversity within populations (H_within), and gene differentiation coefficient (G_ST) is shown in Table 1. From the data displayed in Table 1, the PC1, PC2, PC3, and PC4 primer sets obtained 145, 80, 71, and 91 polymorphic bands, respectively. Of the obtained polymorphic bands, the highest genetic variation was detected in PC2 (N_e = 1.6041, H = 0.3578, H_within = 0.3483, and H_total = 0.3285), whereas PC4 showed the lowest level of genetic diversity (N_e = 1.5414, H = 0.3320, H_within = 0.3210, and H_total = 0.3027).

For the five C. reticulata populations at our five study sites in Yunnan, the total percentage of polymorphic loci (PPL), N_e, H, and I were 85.63%, 1.4917, 0.2937, and 0.4421, respectively, shown in Table 2. Among the five different populations, PPL ranged from 100% to 28.94%, H and I varied from 0.3509 to 0.1199 and from 0.5227 to 0.1750, separately. The highest value of PPL (100%) was detected in the Kunming population with values of H = 0.3402 and I = 0.5145. The lowest value of PPL (28.94%) was found in Chuxiong population with the lowest values of N_e = 1.2046, H = 0.1199, and I = 0.1750. Compared to Kunming, Lijiang possessed a lower percentage of polymorphism loci, but had the highest values of N_e = 1.5895, H = 0.3509, and I = 0.5227.

3.3 | Genetic differentiation within and among populations

Total gene diversity (H_T = 0.3483) was primarily distributed within populations (H_within = 0.3285) on the basis of the genetic variation detected by PC2 as shown in Table 1. With a low G_ST (0.0536), findings indicate that relatively low level genetic differentiation exists between the five populations; rather, the majority of genetic variation is found within populations. Based on the G_ST value, the estimated number of

| Primer code | Primer sets | Polymorphic bands | N_e | H* | I* | H_within | H_total | G_ST | N_m |
|-------------|-------------|-------------------|-----|-----|-----|----------|---------|------|-----|
| PC1         | E-ACT/M-CAC | 145               | 1.5919 | 0.3514 | 0.5289 | 0.3490 | 0.3316 | 0.0475 | 23.5509 |
| PC2         | E-ACC/M-CAA | 80                | 1.6041 | 0.3578 | 0.5369 | 0.3483 | 0.3285 | 0.0536 | 15.2925 |
| PC3         | E3-ACC/M-CTC| 71                | 1.5775 | 0.3449 | 0.5215 | 0.3350 | 0.3204 | 0.0411 | 23.2286 |
| PC4         | E-ACG/M-CTC | 91                | 1.5414 | 0.3320 | 0.5072 | 0.3210 | 0.3027 | 0.0520 | 17.4138 |
| Total       |             | 387               | 1.5787 | 0.3465 | 0.5236 | 0.3397 | 0.3221 | 0.0487 | 20.3415 |

N_e, Effective number of alleles; H, Nei’s (1973) gene diversity; I, Shannon’s information index; H_within, total genetic diversity; H_total, genetic diversity within populations; G_ST, gene differentiation coefficient; N_m, number of migrants.
migrants (N_m) was 15.2925 and indicates that the rate of gene flow is high enough for transferring genetic diversity among populations.

Analysis of molecular variance (AMOVA) on genetic differentiation among and within populations of *C. reticulata* was conducted and is shown in Table 3. Findings from AMOVA revealed that 96.31% of the total genetic variations was contributed by differences within populations (p < .001), which was notably and significantly higher than that among populations (only 3.96% of total genetic variation was due to difference between populations).

### 3.4 Genetic relationships and geographic distances

The genetic distances among all five populations (Kunming, KM; Lijiang, LJ; Chuxiong, CX; Dali, DL; Tengchong, TC) of *C. reticulata* were calculated using TFPGA Software v1.3 and are shown in Table 4. Variation was found in the genetic distance among populations ranging from 0.0077 (KM vs. CX) to 0.0364 (DL vs. LJ) while the geographic distance among populations ranged from 631.5 km to 104.1 km. The highest genetic distance value was 0.361 between KM and LJ with the longest geographic distance 514.5 km, while the lowest genetic distance value 0.0113 was between populations KM and DL with the geographic distance 330.1 km.

#### TABLE 2 Genetic diversity of the five studied populations of *C. reticulata*

| Population | PPL (%) | N_e | H   | I   |
|------------|---------|-----|-----|-----|
| Kunming    | 100     | 1.5695 | 0.3402 | 0.5145 |
| Dali       | 99.74   | 1.5482 | 0.3298 | 0.5006 |
| Lijiang    | 99.74   | 1.5895 | 0.3509 | 0.5227 |
| Tengchong  | 99.74   | 1.5467 | 0.3277 | 0.4979 |
| Chuxiong   | 89.74   | 1.2046 | 0.1199 | 0.1750 |
| Total      | 85.63   | 1.4917 | 0.2937 | 0.4421 |

PPL, Percentage of polymorphic loci; *C. reticulata, Camellia reticulata*.

#### TABLE 3 Analysis of molecular variance (AMOVA) within/among *C. reticulata* populations based on AFLP data

| Source of variation | df  | SSD           | MSD           | Variance component | Total variance (%) | p-Value |
|---------------------|-----|---------------|---------------|--------------------|--------------------|---------|
| Among populations   | 4   | 715.7077      | 178.927       | 2.9541             | 3.69               | <.0010  |
| Within populations  | 189 | 14,177.1812   | 14,007.09     | 77.0499            | 96.31              | <.0010  |
| Total               | 193 | 14,892.8889   | 255.977       | 80.004             | 100                |         |

df, degrees of freedom; SSD, sum of squared deviation; MSD, mean squared deviation; p-Value, Significance tests after 1,000 random permutation; *C. reticulata, Camellia reticulata*.

#### TABLE 4 Geographic distance (km) (above right diagonal) and genetic distance (below left diagonal) among populations of *C. reticulata* based on AFLP analysis

| Pop ID     | Kunming | Dali   | Chuxiong | Tengchong | Lijiang |
|------------|---------|--------|----------|-----------|---------|
| Kunming (KM)| 330.1   | 148.7  | 631.5    | 514.5     |         |
| Dali (DL)  | 0.0113  | 191    | 321.7    | 210.6     |         |
| Chuxiong (CX)| 0.0077 | 0.0123 | 492      | 104.1     |         |
| Tengchong (TC)| 0.0189 | 0.03   | 0.0167   | 550.1     |         |
| Lijiang (LJ)| 0.0361 | 0.0364 | 0.0308   | 0.034     |         |

*C. reticulata, Camellia reticulata.*
semi-double red petals as well as the combination of other types. Many traditional C. reticulata cultivars including Pumencha and Luchengchun are assembled within this group. The green areas represent cultivars mostly collected from Kunming.

Multiple flower characteristics of C. reticulata were differentiated through statistical analyses including flower type, flower color, and blooming period. Twelve representative flower characteristics of C. reticulata were extracted using SPSS’s Component Matrix according to Initial Eigenvalues over 1% (Table S5). About 88.60% of flower data of all samples were covered by these 12 flower characteristics as shown in Table 5. Double-pink-early maturity (20%) is the most common flower pattern of C. reticulata, followed by semi-double-pink-early (17.37%) and double red-middle (14.21%) (Table 5).

Correspondence between the morphological structure and geographical origin is presented in Figure 5. Peach pink is the main flower color for all the five sites. Double flower type is the most type for all sites except for Tengchong with a much higher number of single type. Early and middle blooming period are the two main blooming periods for all sites except for Dali that has fewer varieties with an early blooming period. Regardless of the flower color, flower type, or blooming period, Kunming was found to have the most amount of C. reticulata varieties compared to the other four sites. Therefore, morphological structure seems not to have influence geographical distribution and distances.

3.6 Correlation analysis of genetic structure and morphological structure

Based on the genetic and morphological data, an UPGMA dendrogram was constructed using NTSYSpc-2.11F software (Hasani & Taghavi, 2014) to analyze the genetic structure combined with 12 representative flower characteristics in order to provide a comprehensive evaluation of biodiversity in the C. reticulata accessions (Figure 4). Twelve morphological features were screened according to the importance and use frequency for cultivar classification by local informants. The phylogenetic tree resolved two major groups A and B with a similarity coefficient cutoff 0.545 (Figure 4). Group A is the largest and is composed of two subgroups (one that includes a large proportion of C. reticulata individuals that have double type flowers and the other subgroup separates all C. japonica individuals).
Group B mainly consists of *C. reticulata* cultivars that have pink petals, double type flowers, and early maturity. *C. reticulata* samples in group A had a closer relationship to *C. japonica*, compared to *C. reticulata* samples in group B. This pattern is potentially due to the inclusion of many other *Camellia* species in the formation of *C. reticulata*'s polyploidization that have also contributed to its high

**Figure 4** UPGMA dendrogram based on AFLP data and its relevance with 12 representative morphological characters of *Camellia reticulata* cultivars (the 12 types of flower features were showed in Table 5)
genetic diversity. 

Group A and group B were further clustered into six subgroups with a similarity coefficient cutoff of 0.60, including four subgroups (I, II, III, and IV) in A and two subgroups (V and VI) in B (Figure 4). Cultivars with double type and dark flower petal color, including several samples collected from Tengchong were clustered together in subgroup I. Subgroup II clustered nearly all samples from Tengchong, which was one of the most representative of regional characteristics. Samples of C. reticulata in this cluster were distinctive for pink or white petal color, mostly semi-double and a few double types. Some cultivars with single to semi-double form collected from Laifeng Mountain and E’lu Mountain were placed in the subgroup III. All C. japonica samples were clustered together in subgroup IV and clearly separated from other subgroups of A. Subgroup V was the smallest subgroups comprised of only five cultivars from Kunming, Dali, Chuxiong, and Tengchong. This subgroup was distinctive for its special flower features. Subgroup VI had a complex cluster of cultivars including various petal colors and flower types.

3.7 | Correspondence between genetic diversity and cultivar diversity of C. reticulata

Informants named a total of 75 cultivars of C. reticulata during our ethnobotanical survey and named these cultivars based on their morphological features, local customs, people’s interests, and values. The population of C. reticulata in Kunming, Chuxiong, Dali, and Tengchong all has rich cultivar diversity. However, the populations from Kunming, Dali, and Tengchong have higher genetic diversity than the population of Chuxiong (Figure 6). The cultivars known as “Dalicha,” “Shizitou,” “Zipao,” “Zaotaozhong,” and “Mudan” were found to be very common and popular in Yunnan with a genetic diversity of 0.3298, 0.3402, 0.3298, 0.3402, and 0.3402, respectively. As shown in Figure 5, this study did not have significant correspondence between genetic diversity and cultivar diversity of C. reticulata. Except for the Chuxiong population, C. reticulata in the other three populations showed almost the same genetic diversity among different cultivars.

4 | DISCUSSION

4.1 | Genetic diversity and cultivar diversity of Camellia reticulata

Camellia reticulata is an endemic and religious tree restricted to Yunnan Province in south China. Our AFLP survey of that five natural populations of C. reticulata revealed a relatively high level of genetic diversity at the species level (H = 0.3578, I = 0.5369, PPL = 85.63%; Tables 1 and 2). Interestingly, a large proportion of genetic variation resided within populations (96.31%). By contrast, the genetic diversity of C. reticulata was relatively low between populations (3.69%) (Table 3). Findings showed that geographic distance among five populations was irrelevant to their genetic variations and distances (Figure 2 and Table 4). Therefore, geography is not the driving influence of the genetic diversity within populations of C. reticulata. Rather, the observed genetic diversity of C. reticulata is more likely due to the accumulation of different genotypes resulting from artificial hybridization facilitated by human cultivation practices and other cultural practices that serve to not only protect old cultivars but also to enhance new cultivars.

Comparison of the genetic data of C. reticulata with genetic data collected from populations of close congeners, C. taliensis (Zhao, Yang, Yang, Kato, & Luo, 2014) and C. japonica (Lin, Hu, Ni, Li, & Qiu, 2013), found no variation among wild populations. C. taliensis has a long domestication history as a tea tree widely distributed in Yunnan and has a relatively moderate to high level of overall gene diversity (H_E = 0.597) (Zhao et al., 2014). While no variation was found among wild populations in the AMOVA, most of the variation was detected within populations (70.6% within individuals and 16.5% among individuals within populations) rather than between populations (12.9% of variation was found among populations). Further, C. japonica is recognized to be widely distributed in China with relatively high genetic diversity (PPB: 90.1%; H_E = 0.5013; H = 0.5013) (Lin et al., 2013). While a relatively high level of genetic differentiation among populations of C. reticulata was revealed by AMOVA (22.5%) by this study, relatively low genetic diversity existed within populations. This genetic trend is likely because of overexploitation, frequent human activities, and insufficient conservation management (Lin et al., 2013; Zhao et al., 2014).
Previous studies have supported that a long cultivation history, Buddhist practices, and other traditional cultural factors have contributed to the morphological diversity of *Camellia* trees (Xin et al., 2015). *C. reticulata* has a long history of use for worship and offerings in Buddhism in temples and altars in Yunnan Province by Bai, Yi, and other sociolinguistic groups (Xin et al., 2015). Many ancient trees of *C. reticulata* have been maintained for hundreds of years under the influence of Buddhism in temples and sacred areas in Yunnan (Xin et al., 2015). Other religions and beliefs adhered to the study areas are associated with conservation practices including those linked to nature worship, totemism, and ancestor worship (Long, Zhang, Pei, & Chen, 1999). For example, villages in Chuxiong of Yi Autonomous Prefecture build temples (called “Patron God Monastery”) (Yang & Sun, 2001) that consist of many varieties of *C. reticulata*. In addition, tree worship is one of the most important forms of worship in this area, especially for *C. reticulata* (Liu, Pei, & Chen, 2000). *C. reticulata* has played a role in the life of Yi of Chuxiong as a tribute to worship ancestors as well as for their farming practice (Lai, 2016). The Bai of Dali further regard *C. reticulata* a symbol of ancestral/nature spirits, cultural identity and status and give specific names for each cultivar based on different cultural symbols, meanings, and uses (Xin et al., 2015). For example, in Tengchong, a prized cultivar of *C. reticulata* named Hong-hua-you-cha is considered distinct and highly valued for its seed oil hailed as “Oriental olive oil” that is widely used and commercialized for its high nutrition and medicinal values (Sahari, Ataai, & Hamedi, 2004). Findings from this study support that such cultural practices support rich genetic diversity and cultivar diversity of *C. reticulata* resources. Findings from our ethnobotanical surveys documented the conservation of old trees in temples within the study area and support that cultural beliefs and practices serve to protect old cultivars of *C. reticulata* to a large extent as well as influence their species diversity through natural and human selection and hybridization.

### 4.2 Population genetic structure and morphological structure

In our study, both Nei’s genetic differentiation index among population ($G_{ST} = 0.0536$) and AMOVA (3.69%) values indicated no significant genetic differentiation among the studied populations. The low genetic differentiation exhibited by *C. reticulata* between populations can be explained by ancient economic and cultural exchange including the trade of natural resources such as tree and flower germplasm that has resulted in massive gene flow across ecosystems and populations in Yunnan. For example, the ancient Silk Road fostered economic and cultural exchange between Yunnan Province and its surrounding regions (Wang & Zhao, 2013). The map of the ancient South Silk Road (Figure 4) demonstrates that our study sites in Kunming, Chuxiong, Dali, and Tengchong were located in the important trade arteries and overlap with the distribution of *C. reticulata* in these areas. Except for Chuxiong (PPL = 28.94%; Table 2), all populations in this study were found to have a high genetic diversity at the population level including Kunming (PPL = 100%), Dali (PPL = 99.74%) and Tengchong (PPL = 99.74%). *C. reticulata* also has
rich cultivar diversity in these four populations (Figure 6) with notable variation in flower characteristics (Figure 5). The main flower characteristic include peach pink color, double type petals, and a middle blooming period. These flower characteristics exist in the cultivars of Kunming, Dali, Chuxiong, and Tengchong and indicate that the morphological structure of Camellia reticulata in these four populations is not notably differentiated. This lack of differentiation may be due to economic exchange along the Silk Road that likely fostered the genetic diversity and cultivar diversity of Camellia reticulata. Traders exchanged the cultivars or other Camellia species from different places following their trade routes and play an important role in the high levels of genetic diversity within populations.

Although pollen of Camellia reticulata is dispersed by birds or insects naturally (Kunitake, Hasegawa, Miyashita, & Higuchi, 2004), the mountains in Yunnan where this species occur are distant from each other and have been recognized to result in genetic differentiation among populations (Elstrad, 2014). Mountain geography has been likened to island geography in facilitating divergent evolution and fostering biodiversity (Winger & Bates, 2015). Gene flow through pollen migration by insects is most likely not a driving factor in the ecological evolution process of Camellia reticulata populations, but rather artificial propagation by humans that has been facilitated by cultural exchange and other cultural factors between different geographies.

5 | CONCLUSION

This study highlights that Camellia reticulata resources have relatively high genetic diversity in Yunnan Province of southwestern China and that cultural factors may be a notable driving influence on fostering this diversity compared to geographic distance. AFLP was validated to be useful for examining the genetic evolution of Camellia reticulata as well as for elucidating genetic relationships of different Camellia reticulata cultivars by cluster analysis. This is the first study to provide evidence on the genetic diversity, structure, and differentiation within and among populations of Camellia reticulata. Traditional cultural practices and beliefs of different sociolinguistic groups in the study area of China have likely served an important role in the conservation and enhancement of Camellia reticulata diversity. We expect this study will be helpful for supporting biodiversity conservation, efforts for cultivar introduction, and further studies of Camellia reticulata and related species that are valued by different cultural groups.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

T.X. and W.J.H. wrote the manuscript as co-first authors. C.L.L., J.D.R., and X.T. devised this study. T.X. collected the samples and conducted experiments. T.X. and W.J.H. analyzed the data. S.Z. and J.V.H were involved in the sampling and analyzing data. C.L.L., S.A., and W.J.H revised the manuscript.

DATA ACCESSIBILITY

The AFLP data used to determine the genetic diversity of Camellia are furthermore provided within the Appendix S2.

ORCID

Weijuan Huang http://orcid.org/0000-0003-1181-3667

REFERENCES

Agarwal, M., Shrivastava, N., & Padh, H. (2008). Advances in molecular marker techniques and their applications in plant sciences. Plant Cell Reports, 27, 617–631.

Altieri, M. A. (2004). Linking ecologists and traditional farmers in the search for sustainable agriculture. Frontiers in Ecology and the Environment, 2, 35–42.

Barrett, C. B., Travis, A. J., & Dasgupta, P. (2011). On biodiversity conservation and poverty traps. Proceedings of the National Academy of Sciences of the United States of America, 108, 13907–13912.

Begum, S., AbdEIslam, N. M., Adnan, M., Tariq, A., Yasmin, A., & Hameed, R. (2014). Ethnomedicines of highly utilized plants in the temperate Himalayan region. African Journal of Traditional, Complementary and Alternative Medicines, 11, 132.

Bohn, J. L., Diemont, S. W., Gibbs, J. P., Stehman, S. V., & Vega, J. M. (2014). Implications of Mayan agroforestry for biodiversity conservation in the Calakmul Biosphere Reserve, Mexico. Agroforestry Systems, 88, 269–285.

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., & Kinzig, A. P. (2012). Biodiversity loss and its impact on humanity. Nature, 486, 59–67.

Chen, J., Wang, P., Xia, Y., Xu, M., & Pei, S. (2005). Genetic diversity and differentiation of Camellia sinensis L. (cultivated tea) and its wild relatives in Yunnan province of China, revealed by morphology, biochemical and allozyme studies. Genetic Resources and Crop Evolution, 52, 41–52.

Costa, R., Pereira, G., Garrido, I., Tavares-de-Sousa, M. M., & Espinosa, F. (2016). Comparison of RAPD, ISSR, and AFLP molecular markers to reveal and classify orchardgrass (Dactylis glomerata L.) germplasm variations. PloS One, 11, e0152972.

De Riek, J., De Cock, K., Smulders, M. J., & Nybom, H. (2013). AFLP-based population structure analysis as a means to validate the complex taxonomy of dogroses (Rosa section Caninae). Molecular Phylogenetics and Evolution, 67, 547–559.

Delêtre, M., McKey, D. B., & Hodkinson, T. R. (2011). Marriage exchanges, seed exchanges, and the dynamics of manioc diversity. Proceedings of the National Academy of Sciences of the United States of America, 108, 18249–18254.

Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. Focus, 12, 13–15.

Earl, D. A., & Vonholdt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources, 4, 359–361.
Winger, B. M., & Bates, J. M. (2015). The tempo of trait divergence in geographic isolation: Avian speciation across the Marañon Valley of Peru. Evolution, 69(3), 772–787.

Xin, T., de Riek, J., Guo, H., Jarvis, D., Ma, L., & Long, C. (2015). Impact of traditional culture on Camellia reticulata in Yunnan, China. Journal of Ethnobiology and Ethnomedicine, 11, 74.

Yang, G. C., & Sun, Y. L. (2001). Patron God Monastery: Museum of ethnic Bai culture. Journal of the Central University for Nationalities, 28, 61–69.

Yeh, F., Yang, R., Boyle, T., Ye, Z., & Xiyan, J. (2000). PopGene 32, Microsoft Windows-based freeware for population genetic analysis, version 1.32. Edmonton, AB, Canada: Molecular Biology and Biotechnology Centre, University of Alberta.

Yuan, J. H., Cornille, A., Giraud, T., Cheng, F. Y., & Hu, Y. H. (2014). Independent domestications of cultivated tree peonies from different wild peony species. Molecular Ecology, 23, 82–95.

Zhao, D. W., Yang, J. B., Yang, S. X., Kato, K., & Luo, J. P. (2014). Genetic diversity and domestication origin of tea plant Camellia taliensis (Theaceae) as revealed by microsatellite markers. BMC Plant Biology, 14, 1.

Zhou, S. L., Zou, X. H., Zhou, Z. Q., Liu, J., Xu, C., Yu, J., ... Sang, T. (2014). Multiple species of wild tree peonies gave rise to the ‘king of flowers’, Paeonia suffruticosa Andrews. Proceedings of the Royal Society of London B: Biological Sciences, 281, 1687.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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