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Genetic Diversity of *Carpinus tientaiensis* Cheng, an Endemic and Critically Endangered Species in China, Based on ITS Sequences

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Abstract: The habitat of *Carpinus tientaiensis* Cheng (Betulaceae), an endemic endangered species in China, has been severely damaged, and it is in danger of going extinct. It is of great practical significance to propose corresponding protection measures based on population genetic variation. Based on the nuclear internal transcribed spacer (ITS) sequences, this study discovered that *C. tientaiensis* has relatively high genetic diversity at the species level. At the population level, the genetic variation levels of each population were not consistent, and the genetic diversity of the northern populations was higher than that of the southern populations. There was no significant genetic differentiation and phylogeographic structure among ribotypes and populations. Phylogenetic analysis showed that Sect. *Distegocarpus* and Sect. *Carpinus* were two independent genetic groups among the *Carpinus*, and *C. tientaiensis* may be the more evolutionary species in the Sect. *Carpinus*. Therefore, this study inferred that while *C. tientaiensis* has certain adaptability, climate change and human interference have brought it to an endangered state. Its populations may experienced the bottleneck effect, after which the expansion time was too short, with the populations failing to form a complex genetic structure. In addition, Tiantai Mountain was probably the original community and center of *C. tientaiensis*.

Keywords: *Carpinus tientaiensis*; endangered species; genetic variation; phylogenetic relationship; protective measures

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1. Introduction

Genetic diversity can represent the heritable variation within and between populations of organisms, including plants, and is essential for the conservation, utilization, selection, and improvement of plants [1,2]. When species have high genetic diversity, their adaptability to the environment is stronger, and the lower the diversity, the weaker the adaptability of species to the environment [3,4]. To a certain extent, research on genetic diversity can analyze the origins of species and speculate on the ability and mechanisms of species evolution [3,5]. Therefore, conducting genetic diversity research on endangered or rare species can not only elucidate the origin and evolutionary mechanisms of endangered species, but also the mechanisms by which they become endangered, and provide a theoretical basis for their protection and genetic diversity preservation [6,7].

Genetic diversity has emerged as one of the primary research topic in conservation biology because it is a key component of biodiversity, and biodiversity protection is ultimately about protecting genetic diversity [3]. The internal transcribed spacer (ITS) sequence of ribosomal DNA (rDNA) has the advantages of biparental inheritance, small fragment

and conservative length, high copy number, fast base variation rate, and strong primer versatility [8]. The China Plant BOL (Barcode of Life) Group, based on the research of the CBOL (Consortium for the Barcode of Life) Plant Working Group, having evaluated the universality of DNA barcoding in 6286 individuals of 1757 species in 141 genera of 75 families of seed plants, found that the ITS sequence has high universality in angiosperms, and suggested that it be included in the core barcode for seed plants [9]. Nowadays, the ITS sequence is widely used to study species' genetic diversity, genetic differentiation, and phylogenetic relationships at the inter-genus, inter-species, and even below-species levels [10,11], thus playing an important role in phylogenetic relationships and evolution of plants, as well as in conservation biology [12].

Carpinus tientaiensis Cheng, a deciduous tree of Betulaceae [13–15], is an endemic Tertiary endangered plant [14,15], which is only distributed in Zhejiang Province, China. It is listed on the IUCN (International Union for Conservation of Nature) Red List of Threatened Species as a critically endangered (CR) species [16]. It has important scientific and ecological value in the fields of phytogeography, species evolution, environmental adaptability of plants, and the formation and maintenance of biodiversity. However, its habitat is shrinking and there is a risk of extinction. It is of great practical significance to carry out genetic diversity research on *C. tientaiensis* for its resource protection and utilization. Therefore, this study aims to: (1) analyze the level of genetic variation and the population dynamics of *C. tientaiensis* based on ITS sequences; (2) preliminarily analyze its phylogenetic relationships; (3) propose strategies for the protection and management of germplasm resources.

2. Materials and Methods

2.1. Acquisition of Research Materials

A thorough field survey was conducted in Zhejiang and the adjacent areas between 2018 and 2020, covering all areas where *C. tientaiensis* may be distributed, and the longitude, latitude, and altitude of each population were recorded by handheld GPS. The study collected leaves from all adult individuals in each population, and the collected leaves were immediately stored in a preservation bag containing discolored silicone. In the end, leaf samples of *C. tientaiensis* were collected from Tiantai Mountain (TTS), Dapanshan (DPS), Yangtianhe (YTH), and Shangshantou (SST) (Figure 1; Table 1). The voucher specimens for each population were kept in the landscape experimental training center at Nanjing Forestry University (NFU), Nanjing, China.

Table 1. Collection records and numbers of sequences.

Identifier	Populations	Geographic Location	Longitude	Latitude	Altitude	Number	Species
1-1–1-5	DPS	Dapanshan National Nature Reserve, Pan'an, Zhejiang	120.5218	28.9708	1138	5	*C. tientaiensis*
2-1–2-17	TTS	Tiantai Mountain, Tiantai County, Zhejiang	121.0917	29.2568	901	17	*C. tientaiensis*
3-1–3-2	YTH	Yangtianhe, Qingtian, Zhejiang	119.9907	28.2084	1249	2	*C. tientaiensis*
4-1–4-28	SST	Shangshantou, Jingning She Autonomous County, Zhejiang	119.6320	27.7823	1506	28	*C. tientaiensis*
P1-1	PTSP	Mount Putuo, Zhoushan, Zhejiang	122.3977	30.0177	292	1	*C. putoensis*
P1-2–P1-4	PTSP	Nanjing Forestry University, Nanjing, Jiangsu (introduced from Mount Putuo)	118.8276	32.0852	24	3	*C. putoensis*

Table 1. *Cont.*

Identifier	Populations	Geographic Location	Longitude	Latitude	Altitude	Number	Species
L1-1–L1-3	DPSL	Dapanshan National Nature Reserve, Pan'an, Zhejiang	120.5229	28.9747	1136	3	*C. viminea*
L2-1–L2-6	TTSL	Tiantai Mountain, Tiantai County, Zhejiang	121.0908	29.2525	926	6	*C. viminea*
L3-1–L3-3	YTHL	Yangtianhe, Qingtian, Zhejiang	119.9907	28.2084	1249	3	*C. viminea*
L4-1–L4-5	SSTL	Shangshantou, Jingning She Autonomous County, Zhejiang	119.6309	27.7840	1518	5	*C. viminea*
H1-1–H1-6	TTZH	Tiantangzhai Scenic Spot, Lu'an, Anhui	115.7625	31.1567	760	6	*C. hupeana*
D1-1–D1-6	DPSD	Dapanshan National Nature Reserve, Pan'an, Zhejiang	120.5226	28.9732	1086	6	*C. polyneura*
C1-1–C1-4	TTZC	Tiantangzhai Scenic Spot, Lu'an, Anhui	115.7767	31.1294	1040	4	*C. tschonoskii*
Q1-1–Q1-3	DPSQ	Dapanshan National Nature Reserve, Pan'an, Zhejiang	120.5216	28.9710	1131	3	*C. cordata*

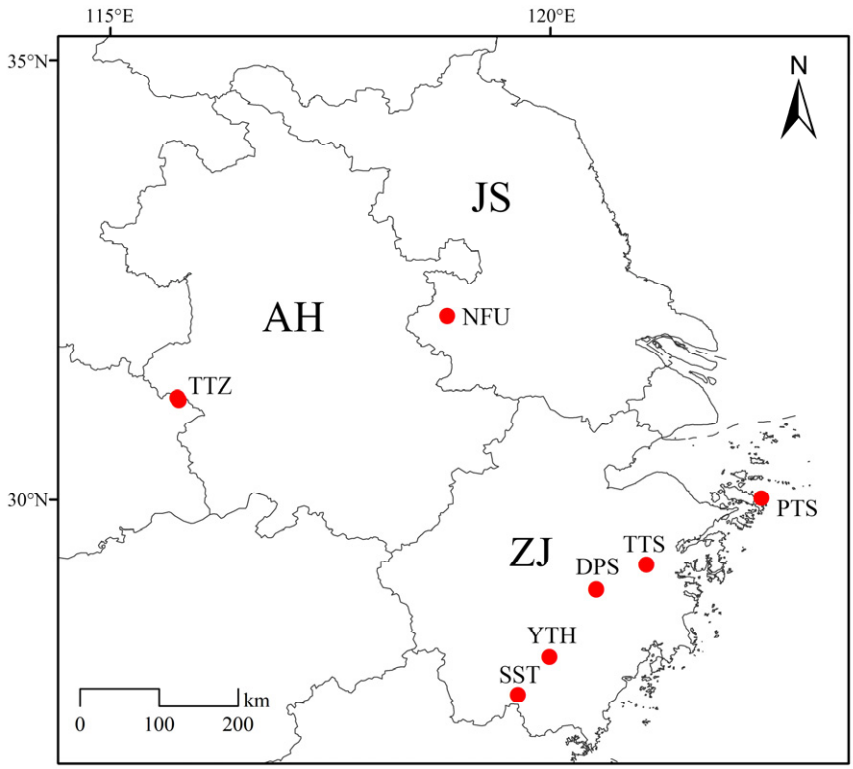

Figure 1. Geographical locations of the sampling sites. JS: Jiangsu Province; AH: Anhui Province; ZJ: Zhejiang Province; NFU: Nanjing Forestry University, Nanjing City; TTZ: Tiantangzhai Scenic Spot, Lu'an City; PTS: Mount Putuo, Zhoushan City; TTS: Tiantai Mountain, Tiantai County; DPS: Dapan Mountain, Pan'an County; YTH: Yangtianhe, Qingtian County; SST: Shangshantou, Jingning She Autonomous County.

2.2. Amplification and Sequencing

The modified CTAB method (modified from [17]) was used to extract the total DNA. PCR amplification was performed on all collected individuals, and the primer sequences [18]

are shown in Table S1. The amplification system was 30 μL and contained 1 μL DNA, 27 μL Tsingke PCR Mix, 1 μL forward and reverse primers with a concentration of 2 μmol/L, and 1 μL template DNA with a concentration of 20 ng/μL. The reaction procedure of amplification was as follows: pre-denaturation at 98 °C for 3 min, followed by amplification for 38 cycles. The reaction system for each cycle was as follows: denaturation at 98 °C for 10 s, followed by primer annealing at 56 °C for 45 s, and then extension at 72 °C for 10 s, and extension at 72 °C for 7 min after all the cycles were completed. The PCR products were detected by electrophoresis, and then the products were sequenced on an ABI 3730 DNA sequencer (Applied Biosystems, Foster, CA, USA) by Tsingke Biotechnology Co., Ltd. (Beijing, China) (www.tsingke.com.cn, accessed on 28 September 2022), and, finally, 52 sequences were obtained (Table 1).

2.3. Analysis of Genetic Diversity

The sequencing results were manually corrected using the SeqMan Pro v11.1.0 software (DNASTAR Inc., Madison, WI, USA), and then all sequences were aligned using MEGA v10.1.7 software [19]. DNASP v5.10 software [20] was used to calculate the ribotype numbers, ribotype diversity (H_d), nucleotide polymorphisms (π), and genetic differentiation coefficients between populations (*GammaSt*), while gene flow (*Nm*) was estimated using the formula $Nm = (1/GammaSt - 1)/2$ [21]. POPART v1.7 software [22] was used to create the median-joining (MJ) network of ribotypes and analyze the relationships between ribotypes. Spatial interpolation analysis of population genetic diversity based on the H_d and IDW (inverse distance weighting) method [23] was performed using ArcGIS v10.2 software (ESRI Inc., Redlands, CA, USA).

PERMUT v2.0 software [24] was used to calculate the total genetic diversity (H_T), the genetic differentiation coefficient between populations (G_{ST} and N_{ST}), and to compare their differences using 10,000 times replacement tests in order to ascertain whether the population had a significant phylogeographic structure. To determine if the population has experienced expansion, Tajima's *D* test, Fu's *Fs* test, and mismatch distribution analysis (MDA) were performed using DNASP v5.10 software [20].

2.4. Phylogenetic Analysis

In order to study the phylogenetic relationships of *C. tientaiensis*, this study also collected other species of *Carpinus*, which are distributed in the same or adjacent areas as *C. tientaiensis*. Due to some individuals sequencing failures, or only 1–2 individuals in certain populations of certain species being successfully sequenced, while populations with less than 3 sequences will be excluded, resulting in a total of 40 sequences being obtained (Table 1). DNASP v5.10 software was used to identify the ribotypes, and then POPART v1.7 software was used to create the MJ network. In addition, for phylogenetic analysis, some ITS sequences were obtained from NCBI (National Center for Biotechnology Information, https://www.ncbi.nlm.nih.gov/, accessed on 1 February 2023), with at least 2 sequences selected for each species, resulting in a total of 10 sequences obtained (Table 2). Phylogenetic analysis was performed using the NJ method (1000 bootstrap replications) in MEGA v10.1.7 software.

Table 2. Species information and GenBank IDs of ITS sequences obtained from the NCBI.

Species	GenBank ID	
C. fangiana	MG569958	MG727560
Ostryopsis davidiana	GQ250099	KC412170
Corylus heterophylla	AF297351	FJ011745
Betula platyphylla	AY761128	FJ011778
Quercus acutissima	AF098428	MN722083

3. Results

3.1. Genetic Diversity and Ribotype Variation

In this study, a total of 52 ITS sequences of *C. tientaiensis* from four locations were obtained, which consisted of 679 bp. A total of nine ribotypes and eight polymorphic sites were detected, including five singleton variable sites and three parsimony informative sites (Table 3). At the species level, the total H_d of *C. tientaiensis* was 0.379 ± 0.086, and the total π was 0.720×10^{-3}. At the population level, the range of H_d of each population was 0 to 0.507 ± 0.140, and the range of π was 0 to 0.840×10^{-3}. Among them, the H_d (0.507 ± 0.140) and π (0.840×10^{-3}) of the TTS population were the highest, while the YTH population had the lowest H_d and π, both of which were 0 (Table 4).

Table 3. Nine ribotypes of *C. tientaiensis* identified based on 5 singleton variable sites and 3 parsimony information sites.

Ribotypes	Variable Sites/bp								Number
	6	26	78	605	612	614	619	641	
R1	T	-	-	-	T	-	-	A	3
R2	T	A	-	-	T	-	-	A	41
R3	T	A	C	G	T	-	-	A	1
R4	T	A	-	-	G	-	-	A	1
R5	-	A	-	-	T	G	-	A	1
R6	T	A	-	-	T	-	-	-	1
R7	-	A	-	-	T	-	-	A	1
R8	T	A	-	G	T	-	-	A	2
R9	T	A	-	-	T	-	C	A	1

Table 4. Ribotype diversity, nucleotide diversity, and ribotype composition of *C. tientaiensis*.

Populations	Ribotype Diversity (H_d)	Nucleotide Diversity ($\pi \times 10^{-3}$)	Ribotype Number	Ribotype Composition and Number
SST	0.331 ± 0.114	0.730	6	R1 (1), R2 (23), R3 (1), R4 (1), R5 (1), R6 (1)
YTH	0.000 ± 0.000	0.000	1	R2 (2)
TTS	0.507 ± 0.140	0.840	5	R1 (2), R2 (12), R7 (1), R8 (1), R9 (1)
DPS	0.400 ± 0.237	0.590	2	R2 (4), R8 (1)

The genetic diversity of other *Carpinus* species collected through the field survey was tested. It was found that at the species level, the total H_d of *C. tschonoskii* was 0.500 ± 0.265, and the total π was 0.730×10^{-3}. The total H_d of *C. polyneura* was 0.333 ± 0.215, and the total π was 0.490×10^{-3}. The total H_d of *C. hupeana* was 0.533 ± 0.172, and the total π was 0.780×10^{-3}. The total H_d of *C. viminea* was 0.221 ± 0.121, and the total π was 0.320×10^{-3}. The total H_d and π of *C. putoensis* and *C. cordata* were 0. At the population level, the H_d (0.533 ± 0.172) and π (0.780×10^{-3}) of the Shangshantou population of *C. viminea* (SSTL) were the highest, and the H_d and π of the other populations were all 0.

3.2. Ribotype Distribution and Network Structure

According to the MJ network of ribotypes of *C. tientaiensis* (Figure 2), there was no obvious genetic differentiation among ribotypes and populations. Among all ribotypes, R2 (41/52 = 78.85%) was the most common ribotype and widely distributed in all populations. It was speculated to be an ancestor ribotype. All other ribotypes had mutated from R2, where R3, R4, R5, and R6 were unique ribotypes of the SST population, and R7 and R9 were unique ribotypes of the TTS population.

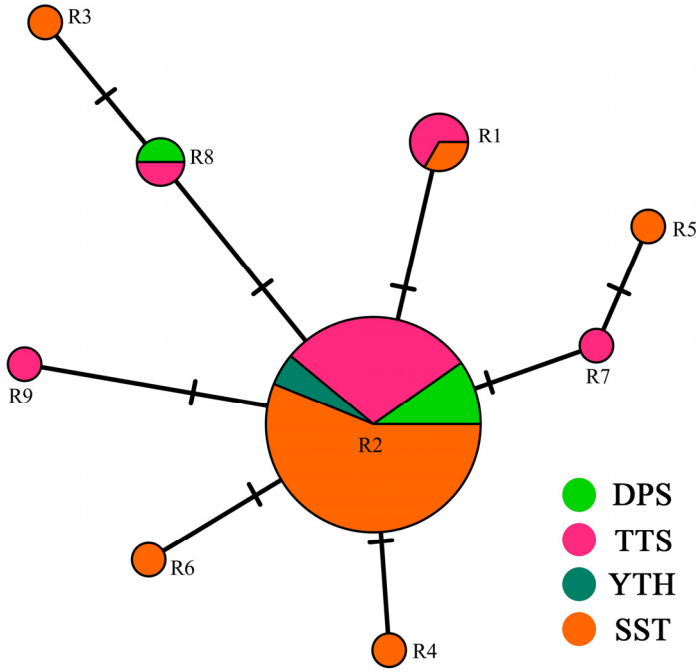

Figure 2. Median-joining (MJ) network of ribotypes of *C. tientaiensis*.

3.3. Gene Flow and Genetic Structure

The gene flow (N_m) between different populations ranged from 4.81463 to 202.75203, with the SST and YTH populations having the most active gene flow, and the YTH and TTS populations having the least gene flow (Table S2; Figure 3). Spatial interpolation analysis revealed that the genetic diversity of the northern population was higher, while that of the southern population was lower (Figure 4). The total H_T of *C. tientaiensis* was 0.309. The genetic differentiation coefficients between populations, G_{ST} and N_{ST}, were −0.001 and −0.016, respectively. Both G_{ST} and N_{ST} were negative values with no statistical significance (p = 0.0988) and N_{ST} values were lower than G_{ST}, indicating that there was no significant phylogeographic structure between populations.

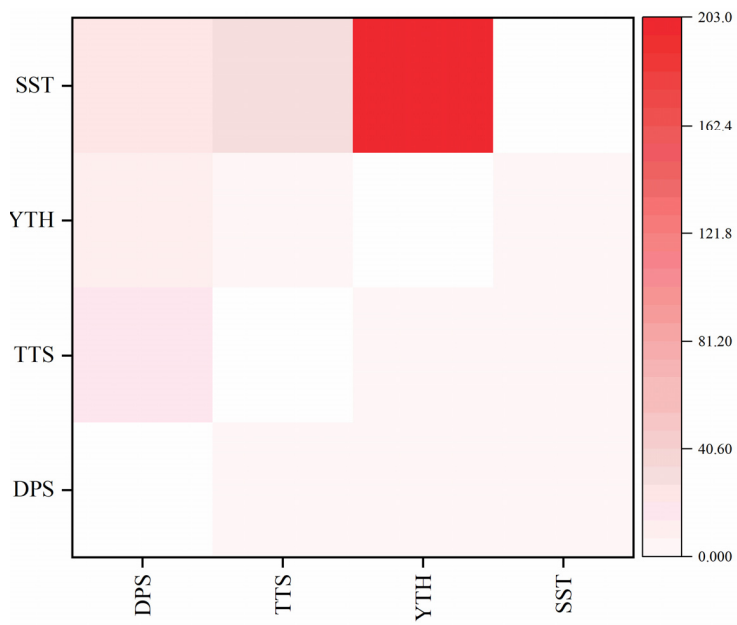

Figure 3. *GammaSt* and *Nm* of *C. tientaiensis*. Above the diagonal is *Nm*, and below the diagonal is *GammaSt*.

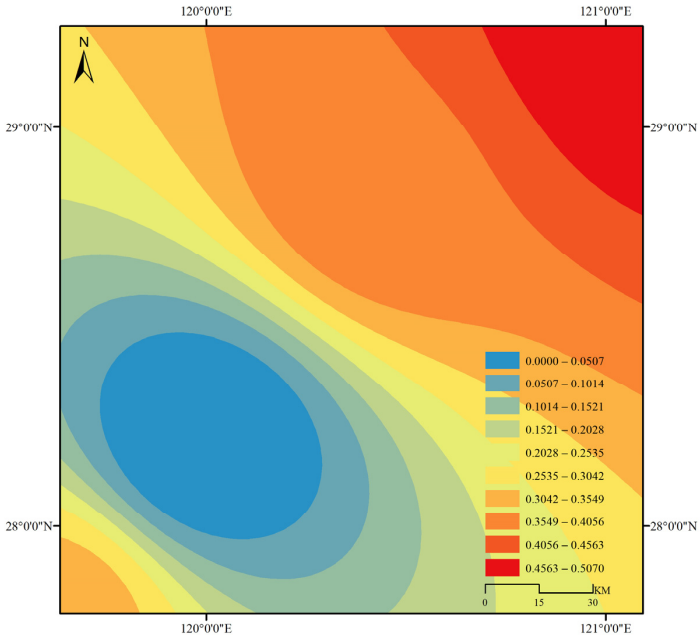

Figure 4. Spatial interpolation analysis of *C. tientaiensis*.

3.4. Population Historical Dynamics

The result of the MDA of *C. tientaiensis* was a single peak (Figure 5), and the neutral test resulted in negative values with no statistical significance (Tajima's $D = -1.09883$ ($p > 0.1$); Fu and Li's $D^* = -1.85254$ ($p > 0.1$)). Therefore, the populations of *C. tientaiensis* may not have experienced large-scale expansion recently.

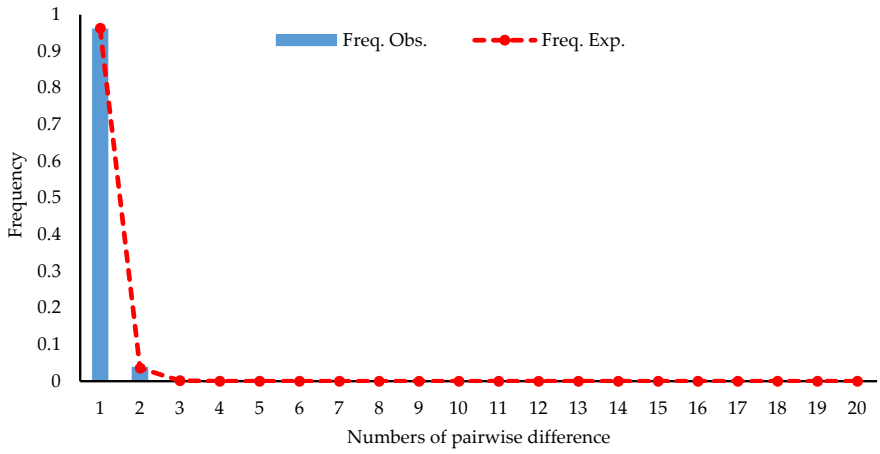

Figure 5. The result of mismatch distribution analysis (MDA) of *C. tientaiensis.*

3.5. Phylogenetic Analysis

In this study, a total of 92 ITS sequences of seven species were obtained, which consisted of 681 bp. Among these sequences, 19 ribotypes and 57 polymorphic sites were identified, including 7 singleton variable sites and 50 parsimony information sites (Table S3). Among them, *C. tientaiensis* has nine ribotypes (R1–R9), *C. putoensis* (R10) and *C. cordata* (R19) each have one ribotype, while *C. tschonoskii* (R11–R12), *C. hupeana* (R13–R14), *C. polyneura* (R15–R16), and *C. viminea* (R17–R18) all have two ribotypes (Table S3; Figure 6).

According to the MJ network (Figure 6), *C. cordata* and *C. viminea* were connected by 16 mutation steps and evolved into genetic branches independently. Therefore, the network supported the conclusion that *Carpinus* can be divided into Sect. *Distegocarpus* and Sect. *Carpinus*. *C. viminea* and mv1 (hypothetical haplotype 1) were connected by six mutation

steps, and *C. hupeana* and *C. polyneura* were connected with mv3 by two mutation steps. It is speculated that *C. hupeana* and *C. polyneura* may have a common ancestor although they both evolved into genetic branches independently. In addition, a total of nine hypothetical haplotypes (mv1–mv9) were detected in six species of Sect. *Carpinus*.

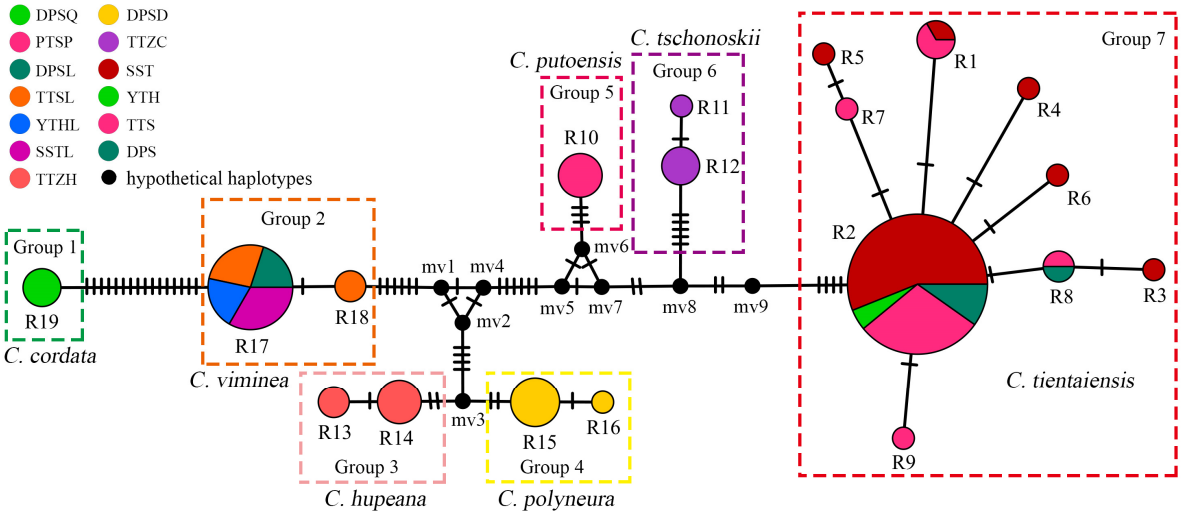

Figure 6. Median-joining (MJ) network based on 19 ribotypes.

Afterward, phylogenetic analysis was conducted using 19 ribotypes and 10 ITS sequences obtained from the NCBI, and the 29 sequences consisted of 617 bp. The phylogenetic results (Figure 7) supported the result of the MJ network (Figure 6). The Sect. *Distegocarpus* and Sect. *Carpinus* independently evolved into two genetic branches. The *C. tientaiensis* had a close genetic relationship with *C. tschonoskii* and *C. putoensis*, while *C. hupeana* and *C. polyneura* had a close genetic relationship.

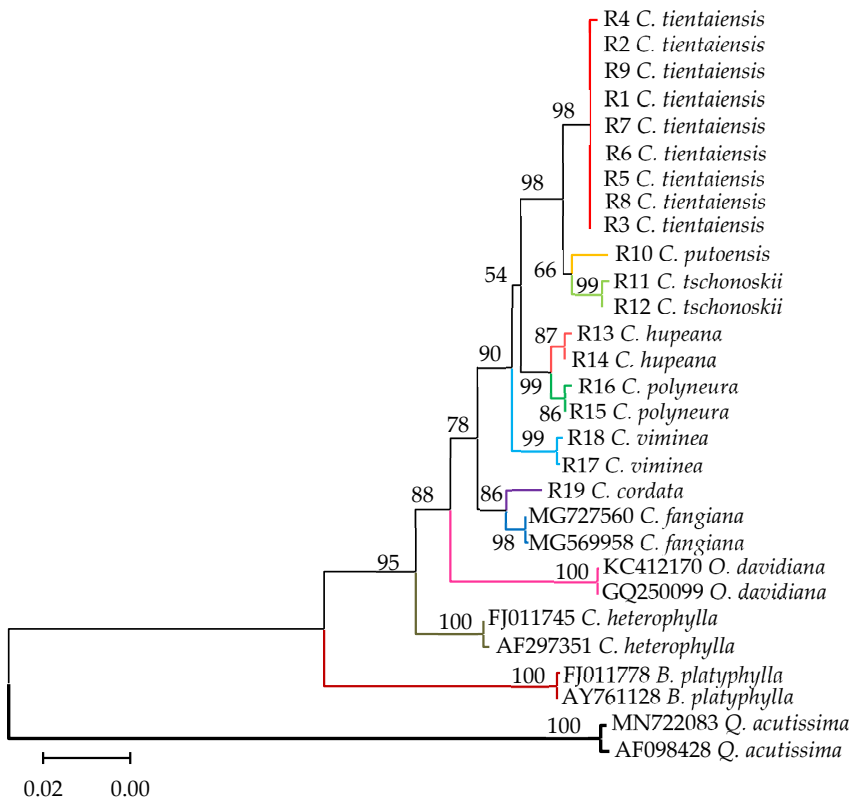

Figure 7. Phylogenetic relationships of 19 ribotypes and 10 ITS sequences obtained from NCBI.

4. Discussion

C. tientaiensis is a unique Tertiary endangered species in China, and its wild populations are only distributed in Zhejiang in eastern China [13–15]. The number of its wild individuals is very small, and the populations are spaced far apart, posing a risk of extinction. Previous study has found that the average value of nuclear gene variation in angiosperm was 0.137 [25]. Based on ITS sequences, it was found that the total genetic diversity (H_T) of *C. tientaiensis* was 0.309, indicating that it had high genetic diversity at the species level. Some studies have shown that some endangered species have lower genetic diversity [26,27], while others have shown that some endangered species have higher genetic variation [28–31]. Researches have shown that the genetic diversity and genetic structure of species were affected by various factors such as distribution range, reproduction mode, evolutionary history, climate, and human interference [32–35], so endangered plants do not necessarily show low genetic diversity. Therefore, it is particularly necessary to identify the genetic diversity of the total and each population, and propose strategies for genetic diversity protection.

The nuclear genes were biparental inheritance [36], and extensive crossing led to genetic recombination, increasing the possibility of genetic variation. There is widespread natural hybridization and infiltration of anemophilous plants [37], and Betulaceae species are typical wind-pollinated plants, which have widespread hybridization and infiltration [38,39]. The same is true of the *Carpinus* species [39–41], which greatly promotes hybridization and gene exchange. *Carpinus* plants are Tertiary species, which have experienced a long evolutionary history. At the same time, *C. tientaiensis* is polyploid [42], which can accommodate and accumulate more variation [43], thus maintaining relatively high genetic diversity. However, study has found that polyploids have a higher mortality rate than diploids [44], and factors such as instability in polyploid genomes, abnormal mitosis and meiosis, and harmful effects of duplicate genes are not conducive to the survival of polyploids [45]. This reminded us of the need to strengthen the protection of polyploid species, especially those in endangered species, to study the ecological adaptability and maintenance mechanisms of polyploid species, and to provide scientific theories and methods for their protection.

The gene flow (*Nm*) among populations of *C. tientaiensis* was between 4.81463 to 202.75203, with a high gene flow. Frequent gene flow may lead to small or even no genetic differentiation or phylogeographic structure between populations [46,47]. The distribution range of *C. tientaiensis* is narrow, and the characteristics of wind pollination and samara spreading easily under the effect of wind have promoted pollination and seed transmission to a certain extent, resulting in a high gene flow. Therefore, there was no significant genetic differentiation and phylogeographic structure between the populations. The MJ network and the coefficient of genetic differentiation among populations (G_{ST} and N_{ST}) also confirm this conclusion. Research has shown that the ancient ribotype is generally located in the center of the ribotype network, with high distribution frequency and wide geographical distribution range [48], so it was speculated that R2 was the ancient ribotype. However, there was no significant genetic differentiation and phylogeographic structure among ribotypes, so it was hypothesized that the populations of *C. tientaiensis* experienced a bottleneck effect, after which the population expanded for a short time, failing to form a complex genetic structure. In addition, due to the existence of the "founder effect", the genetic diversity of the original community was greater than that of the migration diffusion community, so the original community also had more genetic diversity and unique ribotypes [49]. Therefore, Tiantai Mountain may be the original community and center of *C. tientaiensis*. With the dynamic changes in climate, they gradually migrated toward the southwest and eventually spread to other regions.

It is generally believed that *Carpinus* can be divided into Sect. *Distegocarpus* and Sect. *Carpinus* [14,39–41,50], and this study confirmed this conclusion. It was found that *C. viminea* was a diploid species [51], however, *C. tientaiensis* was polyploid, which is the highest ploidy level in Betulaceae [42]. Based on this, it was assumed that among the

species in Sect. *Carpinus*, *C. viminea* may be a relatively original species, and *C. tientaiensis* may be a relatively evolved species. In previous studies on the phylogeny of *Carpinus* based on ITS, it was found that *C. tientaiensis* has a close genetic relationship with *C. viminea*, while *C. hupeana*, *C. tschonoskii*, and *C. putoensis* have a close genetic relationship [52,53]. Recent research has found that *C. tientaiensis* has a close genetic relationship with *C. tschonoskii*, but a distant genetic relationship with *C. viminea* [54]. However, due to the limited number of species and sequences used in these studies, the genetic relationships of *Carpinus* still need further study. As more species were included in the phylogenetic analysis, the genetic relationships of *Carpinus* were further clarified. The study found that *C. tientaiensis* has a close genetic relationship with *C. putoensis* and *C. tschonoskii*, *C. hupeana* has a close genetic relationship with *C. polyneura*, and *C. viminea* has a distant genetic relationship with them [55]. These conclusions were consistent with the results of this research.

It is of great theoretical and practical significance to carry out genetic diversity research on *C. tientaiensis* for its protection and genetic diversity conservation. Tiantai Mountain is a famous scenic spot in China. Its tourism-related construction and sightseeing activities may have a serious impact on the survival of *C. tientaiensis*. Shangshantou is located in a remote and uninhabited area, currently with the largest wild population. Dapanshan is a national nature reserve that has not yet been developed for tourism, but individuals are spaced far apart. The Yangtianhe has built a reservoir, which is likely to damage the original community. This study found that there were differences in genetic diversity among populations, which also reminded us to focus on protecting wild individuals in Dapanshan and Yangtianhe. Therefore, scientific research should be continued to analyze the mechanisms of survival and environmental adaptability of *C. tientaiensis*, and strengthen its maintenance and management.

5. Conclusions

In this study, ITS sequences were used to evaluate the genetic diversity and phylogenetic relationships of *C. tientaiensis*. It was found that there was no obvious genetic differentiation and phylogeographic structure among ribotypes and populations, and R2 was the ancestral ribotype. The populations of *C. tientaiensis* may have experienced a bottleneck effect and failed to form a complex genetic structure, and may not have experienced large-scale expansion recently. There were differences in genetic diversity among populations, with higher genetic diversity in the northern populations and lower genetic diversity in the southern populations. Tiantai Mountain was probably the original community and center of *C. tientaiensis*.

At the nuclear gene level, *C. tientaiensis* had relatively high genetic variation, which indicated that *C. tientaiensis* may have strong environmental adaptability. However, due to human activities such as the construction of scenic spots, dams, and reservoirs, as well as windy and rainy environmental conditions, the fruiting and community regeneration of *C. tientaiensis* were affected, resulting in its endangered status. Therefore, it is necessary to strengthen the protection and management of *C. tientaiensis*, for example, to establish core reserves and carry out artificial breeding, so as to alleviate its habitat threat and endangered status.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14081600/s1, Table S1: Primer sequences and annealing temperature; Table S2: Coefficient of genetic differentiation (*GammaSt*) and gene flow (N_m) of *C. tientaiensis*; Table S3: Nineteen ribotypes and variable sites of seven *Carpinus* species.

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Data Availability Statement: The 19 ribotypes sequences presented in this study have been uploaded to the National Center for Biotechnology Information [https://www.ncbi.nlm.nih.gov/GenBank ID: OR353711 to OR353729]. Publicly available datasets were analyzed in this study, and this data can be found here: [https://www.ncbi.nlm.nih.gov/GenBank ID: MG569958, MG727560, GQ250099, KC412170, AF297351, FJ011745, AY761128, FJ011778, AF098428, MN722083].

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