

**Phylogeography of alpine plant *Parnassia wightiana*
(Celastraceae)**

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1 **Phylogeography of alpine plant *Parnassia wightiana* (Celastraceae)**

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12

13 **Abstract**

14 *Parnassia wightiana*, a perennial herb, is an alpine plant distributed across three
15 biodiversity hotspots in China. This species offers an excellent study system to
16 analyze the distribution pattern and genetic structure of high mountainous plant
17 populations. Three cpDNA regions (*rpl32-trnL*, *trnL-F*, *trnS-G*) from 442 individuals
18 of 39 populations and ITS from 418 individuals of 38 populations were sequenced
19 and analyzed. We identified a total of 26 haplotypes based on the concatenated
20 sequences of cpDNA and a total of 36 based on ITS sequences. A hierarchical
21 analysis of molecular variance (AMOVA) indicated significant phylogeographic
22 structure among populations. Mismatch analysis of multi-peak and Tajima's *D* neutral
23 test demonstrated *P.wightiana* populations within China had not experienced abrupt
24 expansion recently. The allopatric fragmentation resulted in the geographic isolation

due to environmental heterogeneity, producing a high proportion of private haplotypes in the distribution of *P. wightiana* within China.

Keywords

Parnassia wightiana; cpDNA; ITS; genetic diversity; phylogeography

Introduction

Climatic oscillations during the Cenozoic period, especially the Quaternary glacial and interglacial cycle, shaped the distribution of species (Hewitt 2004). Phylogeography is a discipline that deals with the spatial arrangements of genetic lineages, which may explore the genetic diversity and distributions of organisms (Avice 2000). By reconstructing the modern distribution pattern, we can infer how historical events (such as climate) affect the species pattern in the long-term evolution(e.g. Hickerson et al. 2010). For example, different regions may be differentially affected by geotectonic events and climatic fluctuation, and correspondingly respond to climate change differently (Lv and Wu 2009). Herbaceous plants are particularly sensitive to this form of disturbance. The ephemeral nature of herbaceous plants means they may respond more quickly to rapidly changing environments, particularly when compared to tree and shrub species (Comes and Kadereit 1998; Song et al. 2011; Niu et al. 2018). As many alpine plants are under threat from the ongoing climate change (Freeman et al. 2018), comprehending how alpine plants survived past climate change may help us foresee whether and how they will cope with future climate change (Larsson et al. 2022).

Unlike Europe and North America, China was not extensively covered by

massive glacial sheets at the peak of the ice age (e.g. Pinot et al. 1999; Zhou et al. 2004). Consequently, events such as the Qinghai-Tibet Plateau uplift and climatic shifts throughout the Quaternary period likely influenced the genetic structure and distribution pattern of current plants (Yang et al. 2008; Wang et al. 2010; Sun et al. 2011). A steadily increasing number of phylogeographic studies in the Sino-Japanese Floristic Region of mainland China and adjacent areas have documented the population histories of temperate plants in these regions (Qiu et al. 2011).

Parnassia wightiana Wall. ex Wight & Arn. is a perennial herb native to Asia and distributed throughout the Sino-Himalayan region. *Parnassia* are herbaceous dicots distributed across wet and boggy stream-side habitats throughout the Holarctic region. The origin of *Parnassia* can be traced back to the Tertiary period. Based on specimen records and literature review, in addition to China, *P. wightiana* is also distributed in India, Nepal, Bhutan and other countries. The Himalaya-Hengduan region has been inferred to be the center of the distribution and differentiation of *Parnassia* (Phillips 1982; Ku 1987; Wu et al. 2003; Wu 2005; Shu 2017). *P. wightiana* is adapted to both temperate and wet environment, though it is largely restricted to alpine meadows, forest edges and stream banks (Ku 1987; Wu 2005). In the Hengduan Mountains, the species grows primarily in alpine habitats between (1300-) 1500 and 3000 (3200) m. Occasionally, *P. wightiana* can also be found at lower elevations, e.g., along the southern periphery of the Lingnan Region (down to 280 m), or in Xuefeng Mountains on the eastern periphery of Yungui Plateau (310 m). The peripheral alpine populations are generally interpreted as ‘dealpine’ or

‘secondary’ and are founded when seeds or entire plants are transported by mountain streams. Our focal species, *P. wightiana* is distributed across several different elevations in multiple ranges, offering an excellent study system to generalize how paleoclimate may have influenced genetic structure and distribution.

We used three regions of the chloroplast genome (*rpl32-trnL*, *trnL-F*, *trnS-G*) and one nrDNA ITS fragment to investigate the genetic structure and biogeographical history of *P. wightiana*. Our main objectives were to: (1) reveal the genetic variation among and within populations; (2) explore the relationship between estimated historical range changes and climate fluctuations ; and (3) contribute to our expanding understanding of the historical biogeography of alpine plants.

Materials and methods

Population sampling

From July 2016 to September 2020, we collected 601 individuals from 39 populations spanning the entire geographical range of *P. wightiana* of China. Generally, 10-20 individuals were arbitrarily selected from a population, each individual selected 2-3 healthy leaves as experimental materials. The materials collected in the field were dried by silica-gel for DNA extraction. Sampling information for each population is shown in Table 1. Voucher specimens were deposited in the Natural Ecology Museum of Jingdezhen University.

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from the silica-dried leaves using a modified CTAB method (Doyle and Dickson 1987). The nuclear and chloroplast sequences of

all individuals were amplified by PCR using universal primers (Table 2). The PCR reaction system and amplification protocol were identical for both cpDNA and nrDNA. PCR reactions were made using 2.5 µL sample DNA, 0.5 µL TaKaRa Taq DNA polymerase (TaKaRa Inc.), 5 µL 10×Taq Buffer, 5 µL dNTP Mix (2 mM each), 1 µL of each primer in a final reaction volume of 20 µL. The PCR program was as follows: an initial template denaturation at 94°C for 5 min, 35 cycles of 30 second denaturation at 94°C, 1 minute primer annealing at 50°C, a 1.5 min extension at 72°C, and a final extension of 8 min at 72°C. PCR products were isolated and purified using a Gel Extraction Mini Kit (Watson Biotechnologies, Inc.). Purified PCR products were sequenced in both directions with the same PCR primers on an ABI 3730 DNA Sequencer (Applied Biosystems, Inc.).

Population genetic data analyses

ITS and cpDNA sequences were aligned using BioEdit 7.0 (Hall 1999), and saved as a single forward sequence for all individuals. All haplotype sequences detected in this study were deposited in GenBank (<https://www.ncbi.nlm.nih.gov>). The number of haplotypes, haplotype diversity (Hd), and nucleotide diversity (π), and gene flow (Nm) were calculated in DnaSP v6 (Rozas et al. 2017). We also identified polymorphic and information loci. The total genetic diversity (Ht), the average genetic diversity within the population (Hs), as well as the coefficient of genetic differentiation among population (G_{ST} and N_{ST}) were estimated using PERMUT 2.0 with 1,000 random permutations. When N_{st} is larger than G_{st} , closely related haplotypes occur more often in the same area than less closely related haplotypes,

which indicates the significance of phylogeographic structure.

Genealogical relationships among haplotypes were investigated using the median-joining network method as implemented in NETWORK 10.2 (Bandelt et al. 1999). To further quantify genetic differentiation among populations, the hierarchical analyses of molecular variance (AMOVA) was estimated using ARLEQUIN v3.5 (Excoffier and Lischer 2010). Genetic variation both among and within populations were investigated using pairwise differences to evaluate the fixation index (F_{ST}) of haplotype distributions (significance test with 1,000 replicates).

Statistical significance of population expansion was further examined with two neutrality tests, i.e. Tajima's D (Tajima 1989) and Fu's F_s (Fu 1997), as implemented in DNASP, in which significantly negative values indicate population expansion. Mismatch distribution analysis using a spatial expansion model was conducted in ARLEQUIN to infer the historical demography of *P. wightiana* based on both cpDNA and ITS datasets. The shape of the frequency graph of pairwise differences is expected to be multimodal in samples drawn from populations at demographic equilibrium, whereas unimodal distributions are generally found in populations having passed through a recent demographic expansion (Harpending 1994).

Results

Genetic diversity and population differentiation

We obtained 1830bp of cpDNA sequences with 441bp of *rpl32-trnL*, 905bp of *trnL-F*, and 484bp of *trnS-G*. We identified 26 haplotypes defined by 55 polymorphic sites, 19 of which were respectively fixed in a single population (Table 3). Hap1 was

the most widely distributed, and was identified in 13 populations, followed by Hap12(6 populations). Among all 39 populations, 30 were dominated exclusively by a unique haplotype, though both CA and EMS sustained a population of 4 haplotypes, and both FJS and QL each sustained three haplotypes (Fig.1). The results of population genetic analyses showed that, on average, total genetic diversity (Htc), was equal to 0.885, haplotype diversity (Hdc), was 0.880, and nucleotide diversity (πc), was equal to 0.01. Haplotype diversity and nucleotide diversity were highest in the CA population, and were evaluated as 0.718 and 0.005, respectively.

In this study, a total of 418 individuals from 38 populations were sequenced successfully using the ITS fragment. The matrix for aligned ITS had 344 bp with 47 polymorphic sites and 36 haplotypes, 26 of which were respectively fixed in the single population(Table 4). Hap8 and Hap10 were the most abundant haplotypes and were each identified in three populations (Hap8:DMS, HH and ZJJ; Hap10:NC, FJ and SZ). We found that no haplotype was shared by all populations. Among all 38 populations, 31 were dominated exclusively by a unique haplotype , though the FJS and XHY populations each sustained four haplotypes. Similarly, the NC population hosted three haplotypes (Fig.2). The results of population genetic analyses based on the ITS fragment showed that $Htn=0.980$, $Hdn=0.960$, and πn was equal to 0.039. Haplotype diversity and nucleotide diversity were highest in the FJS population, and were evaluated as equal to 0.692 and 0.014, respectively.

Population genetic structure

The coefficients of differentiation measured over the 39 populations were $Gstc=0.868$ and $Nstc=0.949$. Permutation test revealed that $Nstc$ was significantly

higher than *Gstc* ($p<0.05$) indicating the significance influence of phylogeographic structure for the cpDNA haplotypes. This result suggests that closely related haplotypes are likely to exist in the same region. The coefficients of differentiation measured over the 38 populations were $Gstn=0.916$ and $Nstn=0.9457$ based on ITS datasets. Similarly, the permutation test revealed that $Nstn$ was higher than $Gstn$ ($p<0.05$), which indicates the existence of an obvious phylogeographic structure in *P. wightiana*, consistent with the results obtained by cpDNA haplotypes.

The spatial genetic structure of cpDNA and ITS haplotypes was analyzed by AMOVA 1.0 (Dupanloup et al. 2002). AMOVA showed that the genetic variation among populations (cpDNA: 94.97%; ITS: 95.60%) was significantly higher than within populations (cpDNA: 5.03%; ITS: 4.40%). This result suggests that the genetic differentiation of *P. wightiana* is due to obvious isolation among populations rather than variation within populations (Table 5).

Network structure diagrams of each haplotype were obtained by network analysis. The frequency of the chloroplast haplotypes Hap1 and Hap12 were the highest, and analysis placed them in the center of the network structure diagram, implying they may be ancient or progenitor haplotypes (Fig.1). In the nuclear gene sequence haplotype structure diagram, it was found that H8 and H10 were directly related with fewer mutation steps, though most of the haplotypes were far apart, and we found no obvious correspondences between geographical location and haplotype (Fig.2).

Historical dynamics of Population

Whether the population has expanded or not can be inferred by using neutral tests and mismatch distribution analysis. Tajima's D value of cpDNA datasets was 4.18008 ($p < 0.001$), and Fu's F_s 26.853. Tajima's D value of ITS was 1.019 ($p > 0.01$), not significant, and Fu's F_s 4.020. Taken in combination, these results suggest no influence of a demographic expansion on genetic diversity. Mismatch distributions also suggest the species is not undergoing demographic expansion, which is consistent with the results of the neutrality test (Fig. 3).

Discussion

Population genetic diversity and genetic differentiation

The total genetic diversity (H_t) in *P. wightiana* was 0.885 and 0.980 from cpDNA and ITS sequences, higher than the average genetic diversity of angiosperms (Petit et al. 2005). The same result was found on the Tibetan Plateau and adjacent regions in other associated herbaceous species, such as *Primula secundiflora* ($H_{tc}=0.966$; Wang et al. 2008), *Primula poissonii* ($H_{tc}=0.923$; Song et al. 2011), and *Saxifraga egregia* ($H_{tc}=0.868$; Jia et al. 2018). Phylogeographic studies normally cope with multiple populations spanning the entire range, which are potentially more liable to suffer long-term fragmentation and isolation during the cold periods of the Quaternary (Kropf et al. 2003). *Parnassia wightiana* is spread across multiple populations spanning an extremely heterogenous range, the complex topography of the region may have contributed to environmental barriers of gene exchange, leading

to the observed high level of genetic differentiation among populations.

The biogeological history of organisms is also one of the factors affecting species' genetic diversity. In Tertiary relict plants, such as *Alsophila spinulosa* (Su et al. 2005) and *Tetracentron sinense* (Sun et al. 2014), total genetic diversity (H_{tc}) at cpDNA genes is often greater than 0.9. The genus *Parnassia* can be traced back to a similar Tertiary relict, of which *P. wightiana* is an ancestor species distributed across the Sino-Himalayan region (Wu et al. 2003). As cpDNA is transmitted maternally in most plant species (Avice 2000), compared with nr ITS, the lower level of genetic diversity observed on cp DNA gene may be due to the difference in gene transmission (the gene flow of chloroplast DNA fragments is mostly transmitted through seeds, while the gene flow of nuclear genes includes pollen and seeds) (Hu and Li, 2002). Herbaceous plants are generally short and small, and their seed dispersal ability is relatively limited, which may in turn affect the distribution of the studied species (Peti et al. 2003). Based on literature review and field investigation, it was found that the dioecious plant of *P. wightiana* relies on insect pollination (flies are the main floral visitors), and the seeds are basically spread by water flow, with a limited range (Zhang 2020).

AMOVA indicated the genetic variation of *P. wightiana* was largely due to inter-population differences (cpDNA: $F_{st}=0.962$, $p>0.05$; nrITS: $F_{st}=0.961$, $p<0.01$). Similarly, our permutation test revealed that N_{st} was significantly higher than G_{st} ($p<0.05$), which indicates a strong influence of phylogenetic structure for the distribution of both cpDNA and ITS haplotypes. Gene flow (N_m) was demonstrably

low between populations, with the cpDNA and ITS values of 0.03 and 0.02, respectively. We suggest it is likely that geographic isolation and climatic variability caused a decrease in gene exchange between populations resulting in a high level of genetic differentiation among populations. According to our field observations, *P. wightiana* has a high degree of variation in the morphology of degenerated stamens, which also reflects the possibility of a large degree of genetic differentiation between species. Continuing character among populations has now resulted in intermediate types of *P. wightiana* (Ku 1987) which well reflect this regional differentiation (Wu et al. 2003).

Populations historical dynamics of *P. wightiana*

Refugia are areas or regions where a species can survive in unfavorable climate. Generally, they tend to have higher genetic diversity because of preservation of historical diversity and the fact that new alleles arise over time in these isolated pockets. (Petit et al. 2003). High cpDNA genetic diversity was observed in the populations of Emei (EMS) and Qionglai (QL) in Sichuan, Chang'an (CA) in Shaanxi, and Jiangkou (FJS) in Guizhou. Similarly, high ITS genetic diversity was recorded in Jiangkou (FJS) and Kaiyan (XHY) in Guizhou, Changan (CA) in Shaanxi, Huaihua (HH) in Hunan, and both Gongshan (GS) in Yiliang (YL) in Yunnan. The results indicated that high diversity areas of *P. wightiana* were mainly localized in the Hengduan Mountains, Wuling Mountains and Qinling mountains, suggesting the possibility of more than a single refugia. Avise (2000) believed that 'new' haplotypes should form a 'star-like' network with the ancient haplotypes, however, no similar

structure was found in our results. The network diagrams of cpDNA and ITS haplotypes revealed that unique and potentially ancestral haplotypes were largely localized in the Hengduan Mountains of southeastern Qinghai-Tibet Plateau, which may provide multiple glacial refugia within the distribution range. This phylogeographic structure is similar to that observed in some other alpine plants, such as *Eriophyton wallichii* (Wang et al. 2011), *Spiraea alpina* (Zhang et al. 2012), *Rhodiola chrysanthemifolia* (Gao et al. 2016), and *Pritzelago alpina* (Kropf et al. 2003).

The multi-peak curve obtained by the mismatch analysis showed that the *P. wightiana* in our study area did not support the population expansion pattern, and two neutrality tests did not detect obvious evidence of recent population expansion. Dai et al (2022) used MaxEnt model to simulate the suitable distribution area of *P. wightiana* under the climatic conditions of five periods (the last interglacial period, the last glacial period, the middle Holocene, current (1950-2000s) and future(2070s)). The results showed that the distribution area of *P. wightiana* was basically located in the alpine region of China, and did not show obvious range fluctuation. The distribution of each period remained basically stable. Our final analysis is consistent with the results of this study. Our analysis suggests that the observed diversity has little to do with range fluctuations, such as demographic expansion, and instead is likely due to extreme isolation during periods of climate instability during the Quaternary period. Unlike the common pattern of glacial contraction and interglacial or postglacial expansion followed by most temperate

267 plants, *P. wightiana* may have experienced glacial range expansion and interglacial or
268 postglacial range contraction. Differential cycles of range contraction and expansion
269 during the late Quaternary was defined as the “replacement refugia model” (Kropf et
270 al. 2003). The long-distance dispersal hypothesis is thus not likely to be the cause of
271 the disjunct distributions, especially cold-adapted taxa that inhabit high mountains.
272 Therefore, current distribution pattern in *P. wightiana* might be the consequence of
273 topographic isolation and postglacial contraction.

Draft

Conclusion

Geological events and climatic oscillations are important drivers for plant evolution and genetic structure formation. Populations of *P. wightiana* possibly experienced *in situ* altitudinal migrations to response glacial and interglacial intervals, leading to the formation of separate and discrete populations of unique haplotypes. AMOVA revealed high levels of genetic diversity (*H_t*) across all populations, though diversity was largely tied to inter-population variation rather than intra-population diversity among co-occurring haplotypes. This pattern of distribution is associated with similar alpine environments that may have acted as glacial refugia during the Quaternary period.

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Declaration of Competing Interest

The authors declare that they have no competing interests.

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Data Availability

The data generated or analyzed in this study will still be used as the basis for the study of this species in the subsequent stages. We will continue to explore the deep-seated species phylogeographic structure on this basis, but the species distribution data and other published results in the article can be used. Other data, if follow-up academic discussion, can be obtained by contacting the first author.

Draft

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Figure 1. Geographical distribution of the cpDNA haplotypes detected in *P. wightiana* and network of genealogical relationships among 26 haplotypes. Each circle represents a single haplotype with sizes in proportion to frequency.

(Basic map source : <http://bzdt.ch.mnr.gov.cn/> ; Coordinate systems : World Geodetic System 1984)

Figure 2. Geographical distribution of ITS haplotypes detected in *P. wightiana* and network of genealogical relationship among 36 haplotypes. Each circle represents a single haplotype with sizes in proportion to frequency.

(Basic map source : <http://bzdt.ch.mnr.gov.cn/> ; Coordinate systems : World Geodetic System 1984)

Figure 3. Mismatch distribution of *P. wightiana* in the overall populations based on the cpDNA and ITS datasets. Black dots and dashed line show observed values (Obs.), soiled lines indicate expected values (Exp.) under a model of sudden (stepwise) population expansion.

Draft

Table 1 Sampling information of 39 populations of *P. wightiana*

Population code	Location	Longitude/°E	Latitude/°N	Altitude/m	Sample size
LN	Dangchang, Gansu	104.3632	33.5244	2200	18
WY	Weiyuan, Gansu	104.0890	34.9956	2446	21
WX	Wenxian, Gansu	104.4727	33.2804	2100	16
BJ	Baoji, Shanxi	107.0227	34.1748	2102	15
CA	Chang'an, Shanxi	108.4802	33.5028	2098	15
FX	Fengxian, Shanxi	106.5920	34.2089	1750	15
BST	Shangri-La, Yunnan	100.0323	27.5020	2615	18
YL	Yiliang, Yunnan	104.1476	27.4764	1821	15
BC	Binchuan, Yunnan	100.2288	25.3982	2773	15
WH	Lijiang, Yunnan	100.1766	26.9810	3180	13
GS	Gongshan, Yunnan	98.4558	27.7547	2900	15
LS	Lanping, Yunnan	99.4926	26.7886	2588	18
LSL	Lanping, Yunnan	99.5029	26.7579	2388	15
TLB	Shuifu, Yunnan	104.1269	28.4135	1385	16
XHY	Kaiyang, Guizhou	106.9178	26.9509	891	20
FJS	Jiangkou, Guizhou	108.4914	27.4589	408	15
LD	Xuyong, Sichuan	105.2529	27.8980	1302	15
HJ	Gulin, Sichuan	105.4625	28.1216	1340	15
EMS	Emei, Sichuan	103.1754	29.2341	1235	16
QL	Qionglai, Sichuan	103.1244	30.2722	1060	14
FJ	Fengjie, Chongqing	109.2704	30.4048	1306	15
HSZ	Shizhu, Chongqing	108.4690	30.1660	1500	15
NC	Nanchuan, Chongqing	107.1106	29.0233	2098	15
SNJ	Shennongjia, Hubei	110.1560	31.2664	2911	16
HH	Huaihua, Hunan	110.0251	27.3227	310	15
CB	Chengbu, Hunan	110.1214	26.1092	1031	15
SZ	Sangzhi, Hunan	110.0461	29.4605	1469	16
ZJJ	Zhangjiajie, Hunan	110.2731	29.2206	917	17
SH	Shanghang, Fujian	116.9233	25.3266	700	16
NJ	Nanjing, Fujian	117.1407	24.5398	755	15
LG	Lingui, Guangxi	109.5734	25.3627	803	11
LJY	Xing'an, Guangxi	110.4289	25.8147	481	15
DMS	Wuming, Guangxi	108.4347	23.5070	1340	15
CZ	Chaozhou, Guangdong	116.5850	23.8574	283	13
LM	Longmen, Guangdong	113.5318	23.3813	470	16
YS	Yangshan, Guangdong	112.5318	24.4610	690	13
MT	Motuo, Xizang	95.00527	29.4661	3155	12
ZC	Jilong, Xizang	85.3766	28.3939	3109	16
JP	Jilong, Xizang	85.3275	28.3775	2740	15

Table 2 Primers selected in this study

Genotype	Gene fragment	Primer name	Primers sequences(5'-3')	References
cpDNA	rpl32-trnL	rpL32-F	CAGTTCCAAAAAACGTACTTC	(Shaw et al. 2007)
		trnL-UAG	CTGCTTCCTAAGAGCAGCGT	
	trnS-G	trnS-GCU	AACTCGTACAACGGATTAGCAAT	(Taberlet 1991)
			C	
	trnL-F	trnG-UUC	GAATCGAACCCGCATCGTTAG	(Hamilton 1999)
		trnL	CGAAATCGGTAGACGCTACG	
nDNA	ITS	trnF	ATTTGAACTGGTGACACGAG	(Wang 2012)
		ITS5	GGAAGTAAAAGTCGTAACAAGG	
		ITS4	TCCTCCGCTTATTGATATGC	

Draft

Table 3 Haplotype distribution and diversity of cpDNA sequence

Code	Number	Haplotype (Number)	Haplotype number	Haplotype diversity	Nucleotide diversity	Private haplotype number
BC	5	H1	1	0	0	0
BJ	9	H1	1	0	0	0
BST	20	H2	1	0	0	0
CA	13	H3(6)、H4(4)、H5(2)、H6(1)	4	0.718	0.005	H3、H4、H5、H6
CB	10	H1(5)、H7(5)	2	0.556	0.005	H7
CZ	10	H8	1	0	0	0
DMS	10	H1	1	0	0	0
EMS	14	H9(1)、H10(1)、H11(1)、H12(11)	4	0.396	0.002	H9、H10、H11
FJ	12	H1	1	0	0	0
FJS	10	H1(8)、H13(1)、H14(1)	3	0.378	0.002	H13、H14
FX	10	H1	1	0	0	0
GS	7	H15	1	0	0	0
HH	8	H1(7)、H16(1)	2	0.250	0.002	0
HJ	10	H12	1	0	0	0
HSZ	10	H17	1	0	0	H17
JP	12	H15(9)、H18(3)	2	0.409	0.002	H18
LD	14	H12	1	0	0	0
LG	9	H1(2)、H16(7)	2	0.389	0.003	0
LJY	11	H1	1	0	0	0
LM	7	H8	1	0	0	0
LN	11	H19	1	0	0	0
LS	18	H2	1	0	0	0
LSL	18	H20	1	0	0	H20
MT	12	H15	1	0	0	0
NC	7	H1	1	0	0	0
NJ	10	H8	1	0	0	0
QL	13	H21(4)、H22(7)、H23(2)	3	0.641	0.003	H21、H22、H23
SH	13	H8	1	0	0	0
SNJ	17	H1	1	0	0	0
SZ	11	H12	1	0	0	0
TLB	17	H12	1	0	0	0
WH	11	H2	1	0	0	0
WX	7	H19	1	0	0	0
WY	11	H19(6)、H24(5)	2	0.545	0.0003	H24
XHY	21	H25	1	0	0	H25
YL	9	H12	1	0	0	0
YS	10	H8	1	0	0	0
ZC	8	H15	1	0	0	0
ZJJ	7	H1(1)、H26(6)	1	0	0	H26
Total	442		26	0.880	0.012	19

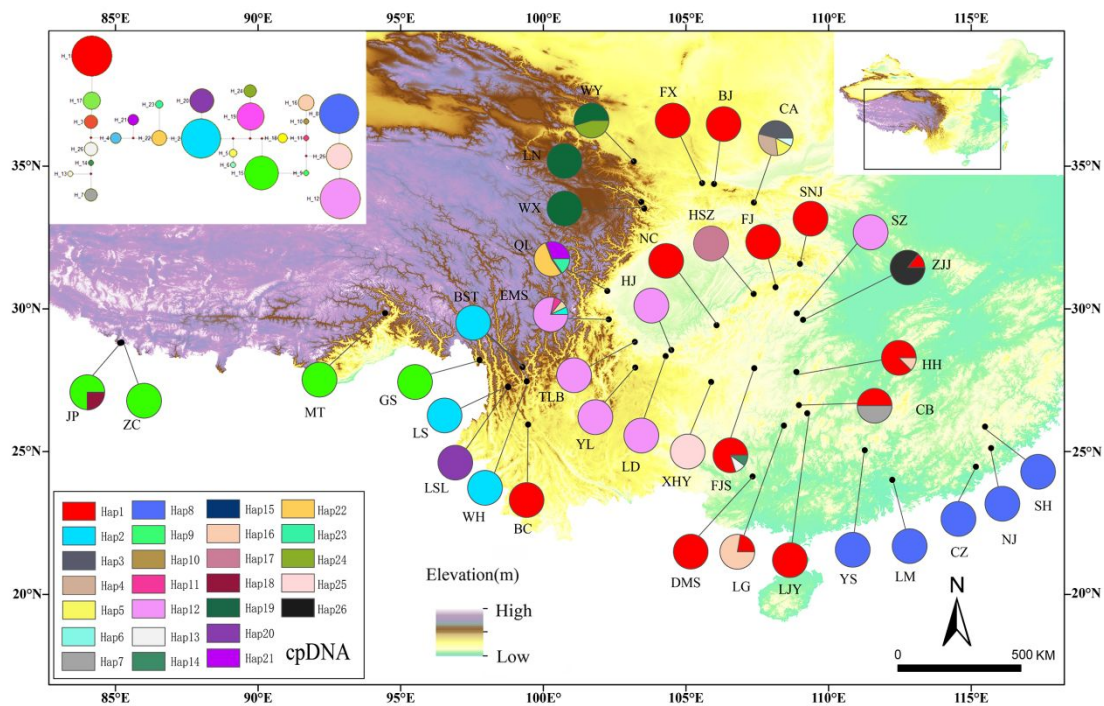
Table 4 Haplotype distribution and diversity of ITS sequence

Code	Number	Haplotype (Number)	Haplotype number	Haplotype diversity	Nucleotide diversity	Private haplotype number
BC	5	H1	1	0	0	H1
BJ	19	H2	1	0	0	0
BST	20	H3	1	0	0	H3
CA	11	H4(9)、H5(2)	2	0.328	0.011	H5
CB	11	H6	1	0	0	0
CZ	11	H7	1	0	0	0
DMS	10	H8	1	0	0	0
EMS	14	H9	1	0	0	0
FJ	6	H10	1	0	0	0
FJS	13	H11(2)、H12(2)、H13(2)、H14(7)	4	0.692	0.014	H11、H12、H13、H14
FX	5	H2	1	0	0	0
GS	14	H15(2)、H16(12)	2	0.264	0.008	H15、H16
HH	6	H4(1)、H8(5)	2	0.333	0.014	0
HJ	10	H9	1	0	0	0
HSZ	5	H17	1	0	0	H17
JP	10	H18	1	0	0	0
LD	14	H19	1	0	0	0
LG	10	H20	1	0	0	H20
LJY	11	H6	1	0	0	0
LN	5	H21	1	0	0	0
LS	16	H22	1	0	0	0
LSL	14	H22	1	0	0	0
MT	12	H23	1	0	0	H23
NC	9	H10(2)、H24(5)、H25(2)	3	0.667	0.011	H24、H25
NJ	12	H26	1	0	0	H26
QL	12	H27	1	0	0	H27
SH	12	H7	1	0	0	0
SNJ	6	H28	1	0	0	H28
SZ	7	H10	1	0	0	0
TLB	17	H29	1	0	0	H29
WH	11	H30	1	0	0	H30
WX	10	H21	1	0	0	0
WY	7	H21	1	0	0	0
XHY	21	H31(15)、H32(2)、H33(3)、H34(1)	4	0.481	0.006	H31、H32、H33、H34
YL	10	H19(8)、H35(2)	2	0.356	0.001	H35
YS	10	H36	1	0	0	H36
ZC	14	H18	1	0	0	0
ZJJ	8	H8	1	0	0	0
Total	418		36	0.960	0.039	25

Table 5 Analyses of molecular variance (AMOVA) in *P. wightiana*

Source of variation	<i>df</i>	Sum of squares	Variance components	Percentage of variation %
cpDNA				
Among populations	38	4603.804	10.67003 Va	94.97
Within populations	403	227.843	0.56537 Vb	5.03
Total	441	4831.647	11.23540	
ITS				
Among populations	37	2705.260	6.64363 Va	95.60
Within populations	380	116.202	0.30580 Vb	4.40
Total	417	2821.462	6.94943	

Notes: Fixation index (*F_{st}*) of cpDNA is 0.962; Fixation index (*F_{st}*) of ITS is 0.961



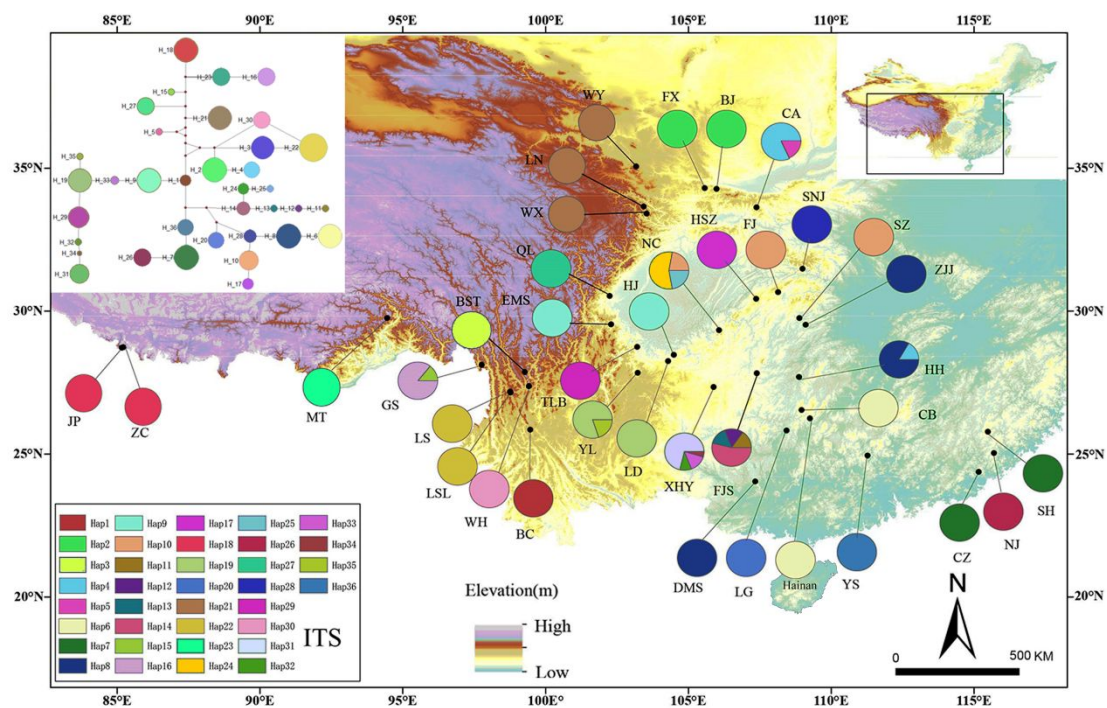


Figure 2
6372×4139mm(300×300 DPI)

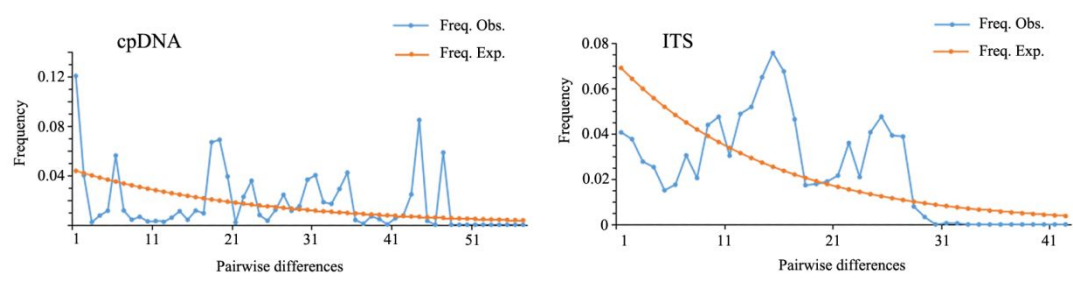


Figure 3
1772×586mm(300×300 DPI)

Table 1 Sampling information of 39 populations of *P. wightiana*

Population code	Location	Longitude/°E	Latitude/°N	Altitude/m	Sample size
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FX	Fengxian, Shanxi	106.5920	34.2089	1750	15
BST	Shangri-La, Yunnan	100.0323	27.5020	2615	18
YL	Yiliang, Yunnan	104.1476	27.4764	1821	15
BC	Binchuan, Yunnan	100.2288	25.3982	2773	15
WH	Lijiang, Yunnan	100.1766	26.9810	3180	13
GS	Gongshan, Yunnan	98.4558	27.7547	2900	15
LS	Lanping, Yunnan	99.4926	26.7886	2588	18
LSL	Lanping, Yunnan	99.5029	26.7579	2388	15
TLB	Shuifu, Yunnan	104.1269	28.4135	1385	16
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FJS	Jiangkou, Guizhou	108.4914	27.4589	408	15
LD	Xuyong, Sichuan	105.2529	27.8980	1302	15
HJ	Gulin, Sichuan	105.4625	28.1216	1340	15
EMS	Emei, Sichuan	103.1754	29.2341	1235	16
QL	Qionglai, Sichuan	103.1244	30.2722	1060	14
FJ	Fengjie, Chongqing	109.2704	30.4048	1306	15
HSZ	Shizhu, Chongqing	108.4690	30.1660	1500	15
NC	Nanchuan, Chongqing	107.1106	29.0233	2098	15
SNJ	Shennongjia, Hubei	110.1560	31.2664	2911	16
HH	Huaihua, Hunan	110.0251	27.3227	310	15
CB	Chengbu, Hunan	110.1214	26.1092	1031	15
SZ	Sangzhi, Hunan	110.0461	29.4605	1469	16
ZJJ	Zhangjiajie, Hunan	110.2731	29.2206	917	17
SH	Shanghang, Fujian	116.9233	25.3266	700	16
NJ	Nanjing, Fujian	117.1407	24.5398	755	15
LG	Lingui, Guangxi	109.5734	25.3627	803	11
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DMS	Wuming, Guangxi	108.4347	23.5070	1340	15
CZ	Chaozhou, Guangdong	116.5850	23.8574	283	13
LM	Longmen, Guangdong	113.5318	23.3813	470	16
YS	Yangshan, Guangdong	112.5318	24.4610	690	13
MT	Motuo, Xizang	95.00527	29.4661	3155	12
ZC	Jilong, Xizang	85.3766	28.3939	3109	16
JP	Jilong, Xizang	85.3275	28.3775	2740	15

Table 2 Primers selected in this study

Genotype	Gene fragment	Primer name	Primers sequences(5'-3')	References
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		trnL-UAG	CTGCTTCCTAAGAGCAGCGT	
	trnS-G	trnS-GCU	AACTCGTACAACGGATTAGCAAT	(Taberlet 1991)
			C	
	trnL-F	trnG-UUC	GAATCGAACCCGCATCGTTAG	(Hamilton 1999)
		trnL	CGAAATCGGTAGACGCTACG	
nDNA	ITS	trnF	ATTTGAACTGGTGACACGAG	(Wang 2012)
		ITS5	GGAAGTAAAAGTCGTAACAAGG	
		ITS4	TCCTCCGCTTATTGATATGC	

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Table 3 Haplotype distribution and diversity of cpDNA sequence

Code	Number	Haplotype (Number)	Haplotype number	Haplotype diversity	Nucleotide diversity	Private haplotype number
BC	5	H1	1	0	0	0
BJ	9	H1	1	0	0	0
BST	20	H2	1	0	0	0
CA	13	H3(6)、H4(4)、H5(2)、H6(1)	4	0.718	0.005	H3、H4、H5、H6
CB	10	H1(5)、H7(5)	2	0.556	0.005	H7
CZ	10	H8	1	0	0	0
DMS	10	H1	1	0	0	0
EMS	14	H9(1)、H10(1)、H11(1)、H12(11)	4	0.396	0.002	H9、H10、H11
FJ	12	H1	1	0	0	0
FJS	10	H1(8)、H13(1)、H14(1)	3	0.378	0.002	H13、H14
FX	10	H1	1	0	0	0
GS	7	H15	1	0	0	0
HH	8	H1(7)、H16(1)	2	0.250	0.002	0
HJ	10	H12	1	0	0	0
HSZ	10	H17	1	0	0	H17
JP	12	H15(9)、H18(3)	2	0.409	0.002	H18
LD	14	H12	1	0	0	0
LG	9	H1(2)、H16(7)	2	0.389	0.003	0
LJY	11	H1	1	0	0	0
LM	7	H8	1	0	0	0
LN	11	H19	1	0	0	0
LS	18	H2	1	0	0	0
LSL	18	H20	1	0	0	H20
MT	12	H15	1	0	0	0
NC	7	H1	1	0	0	0
NJ	10	H8	1	0	0	0
QL	13	H21(4)、H22(7)、H23(2)	3	0.641	0.003	H21、H22、H23
SH	13	H8	1	0	0	0
SNJ	17	H1	1	0	0	0
SZ	11	H12	1	0	0	0
TLB	17	H12	1	0	0	0
WH	11	H2	1	0	0	0
WX	7	H19	1	0	0	0
WY	11	H19(6)、H24(5)	2	0.545	0.0003	H24
XHY	21	H25	1	0	0	H25
YL	9	H12	1	0	0	0
YS	10	H8	1	0	0	0
ZC	8	H15	1	0	0	0
ZJJ	7	H1(1)、H26(6)	1	0	0	H26
Total	442		26	0.880	0.012	19

Table 4 Haplotype distribution and diversity of ITS sequence

Code	Number	Haplotype (Number)	Haplotype number	Haplotype diversity	Nucleotide diversity	Private haplotype number
BC	5	H1	1	0	0	H1
BJ	19	H2	1	0	0	0
BST	20	H3	1	0	0	H3
CA	11	H4(9)、H5(2)	2	0.328	0.011	H5
CB	11	H6	1	0	0	0
CZ	11	H7	1	0	0	0
DMS	10	H8	1	0	0	0
EMS	14	H9	1	0	0	0
FJ	6	H10	1	0	0	0
FJS	13	H11(2)、H12(2)、H13(2)、H14(7)	4	0.692	0.014	H11、H12、H13、H14
FX	5	H2	1	0	0	0
GS	14	H15(2)、H16(12)	2	0.264	0.008	H15、H16
HH	6	H4(1)、H8(5)	2	0.333	0.014	0
HJ	10	H9	1	0	0	0
HSZ	5	H17	1	0	0	H17
JP	10	H18	1	0	0	0
LD	14	H19	1	0	0	0
LG	10	H20	1	0	0	H20
LJY	11	H6	1	0	0	0
LN	5	H21	1	0	0	0
LS	16	H22	1	0	0	0
LSL	14	H22	1	0	0	0
MT	12	H23	1	0	0	H23
NC	9	H10(2)、H24(5)、H25(2)	3	0.667	0.011	H24、H25
NJ	12	H26	1	0	0	H26
QL	12	H27	1	0	0	H27
SH	12	H7	1	0	0	0
SNJ	6	H28	1	0	0	H28
SZ	7	H10	1	0	0	0
TLB	17	H29	1	0	0	H29
WH	11	H30	1	0	0	H30
WX	10	H21	1	0	0	0
WY	7	H21	1	0	0	0
XHY	21	H31(15)、H32(2)、H33(3)、H34(1)	4	0.481	0.006	H31、H32、H33、H34
YL	10	H19(8)、H35(2)	2	0.356	0.001	H35
YS	10	H36	1	0	0	H36
ZC	14	H18	1	0	0	0
ZJJ	8	H8	1	0	0	0
Total	418		36	0.960	0.039	25

Table 5 Analyses of molecular variance (AMOVA) in *P. wightiana*

Source of variation	<i>df</i>	Sum of squares	Variance components	Percentage of variation %
cpDNA				
Among populations	38	4603.804	10.67003 Va	94.97
Within populations	403	227.843	0.56537 Vb	5.03
Total	441	4831.647	11.23540	
ITS				
Among populations	37	2705.260	6.64363 Va	95.60
Within populations	380	116.202	0.30580 Vb	4.40
Total	417	2821.462	6.94943	

Notes: Fixation index (*F_{st}*) of cpDNA is 0.962; Fixation index (*F_{st}*) of ITS is 0.961

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Figure 1. Geographical distribution of the cpDNA haplotypes detected in *P. wightiana* and network of genealogical relationships among 26 haplotypes. Each circle represents a single haplotype with sizes in proportion to frequency.

Figure 2. Geographical distribution of ITS haplotypes detected in *P. wightiana* and network of genealogical relationship among 36 halpotypes. Each circle represents a single haplotype with sizes in proportion to frequency.

Figure 3. Mismatch distribution of *P. wightiana* in the overall populations based on the cpDNA and ITS datasets. Black dots and dashed line show observed values (Obs.), soiled lines indicate expected values (Exp.) under a model of sudden (stepwise) population expansion.

Draft

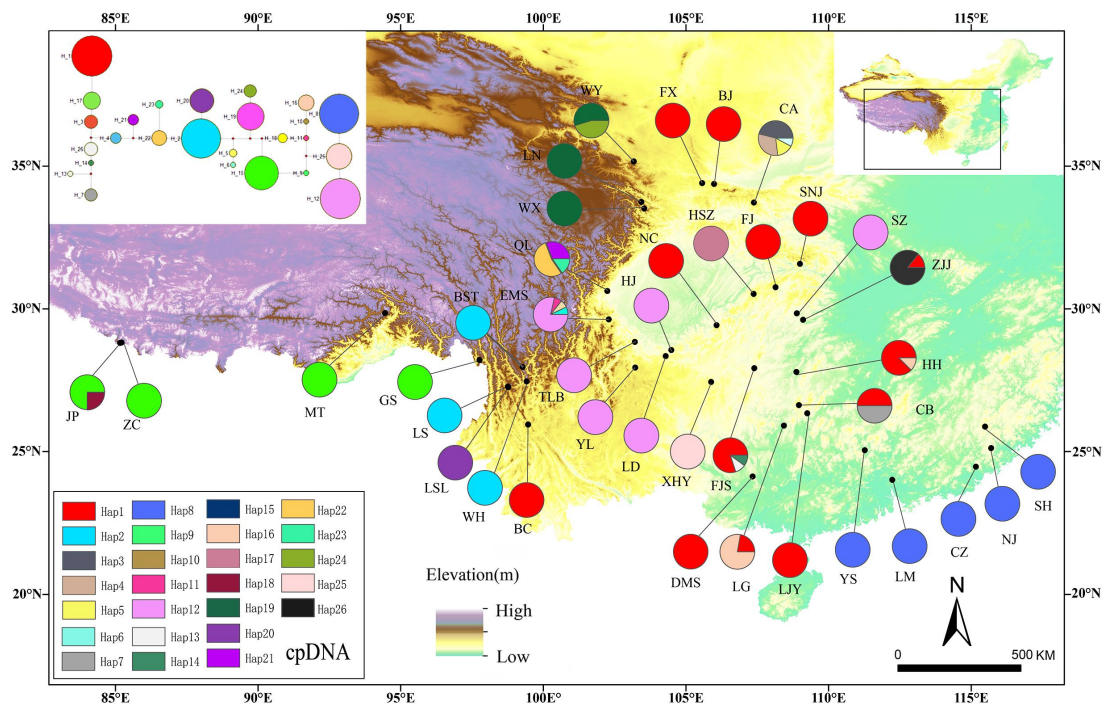


Figure 1
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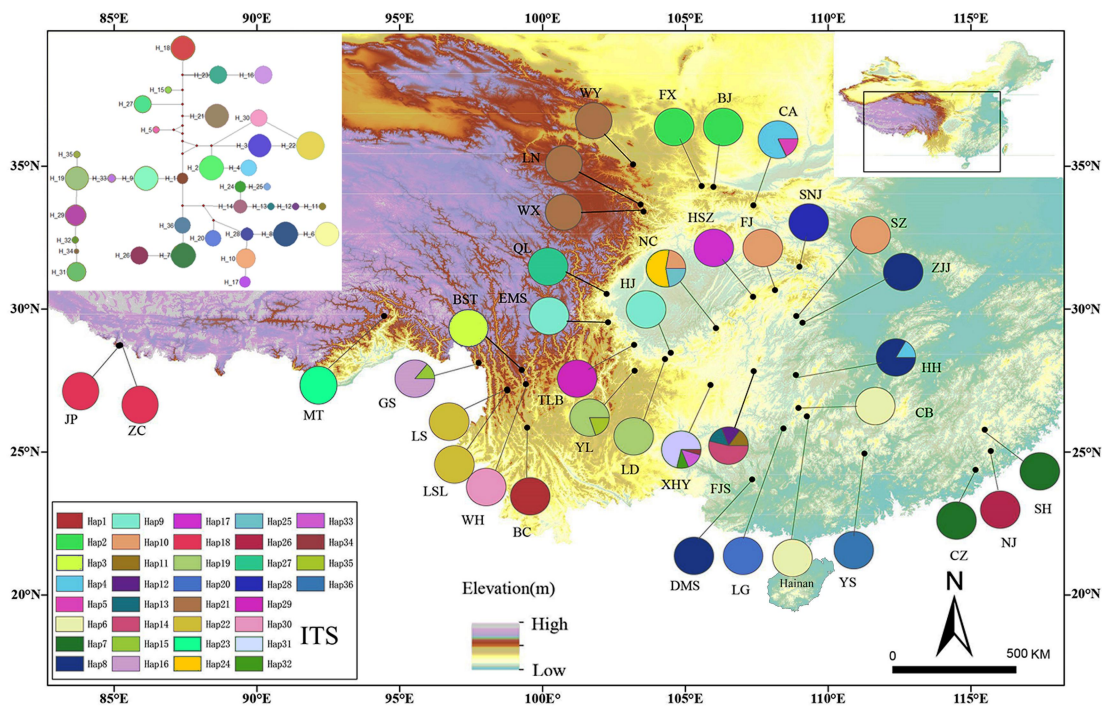


Figure 2

6372×4139mm(300×300 DPI)

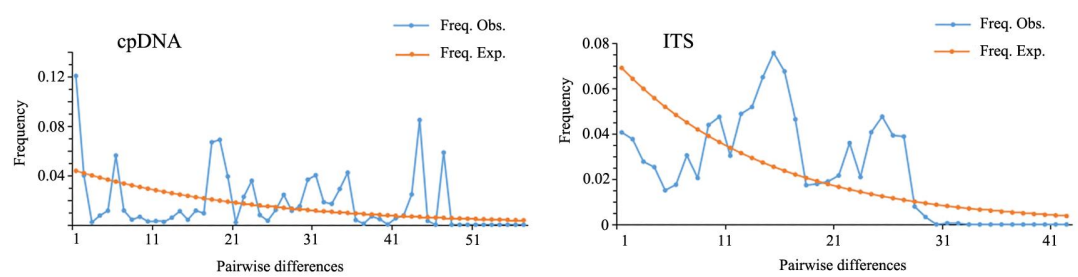


Figure 3
1772×586mm(300×300 DPI)